Studies to Determine the Cause of Mortality in Alevins of Chinook Salmon *(Oncorhynchus tshawytscha)* at Chehalis Hatchery, British Columbia

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STUDIES TO DETERMINE THE CAUSE OF MORTALITY IN ALEVINS OF CHINOOK SALMON (Oncorhynchus tshawytscha) AT CHEHALIS HATCHERY, BRITISH COLUMBIA

by

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ABSTRACT

Alderdice, D. F. and D. R. Harding. (Editors). 1987. Studies to determine the cause of mortality in alevins of chinook salmon (Oncorhynchus tshawytscha) at Chehalis Hatchery, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1590: 96 p.

The Chehalis Hatchery, located on the Chehalis River, a tributary to the Harrison River in the upper Fraser Valley, British Columbia, began operations in 1982. Several species of Pacific salmon are incubated as eggs and cultured there, including the chinook salmon. From the onset of operations the chinook alevins have been subject to heavy mortality beginning several weeks after hatching. Origin of the chinook hatchery stock is the nearby Harrison River. A continuing pathological investigation has been conducted since the problem was first observed. As no pathogen could be identified with the problem, other lines of investigation were brought in, including a consideration of genetic susceptibility of Harrison River and other compared stocks, and an examination of the influence of the rather "soft" Chehalis Hatchery water supply on response of alevins to ionic additions to the incubation water (Ca^{2+}, Na^{+}, K^{+}). To date no pathogenic agent responsible for the problem has been found. Continuing investigation, however, has shown that the causal agent must be a pathogen. Genetic studies show that the Harrison River stock is particularly susceptible to the problem, and that susceptibility is transmitted largely by the Harrison River male. It is interesting to note that the Harrison River chinook stock consists largely (about 98%) of "white-fleshed" chinook (white spring salmon). In comparison, "red-fleshed" chinook (red spring salmon) from the Capilano River (formerly Big Qualicum River stock) show a lower susceptibility to the problem. Salmonids are known to show an increased incidence of physiological and pathological problems in soft waters in which the concentrations of Ca^{2+}, Na^{+}, or K^{+} are low. Additions of these ions to the incubation water supply show that increased concentrations of Ca^{2+} and Na^{+} have a significant effect on reducing mortality caused by the presumed pathogen. Nevertheless, mortality rates in waters dosed with Ca^{2+} and Na^{+} at the Chehalis Hatchery remain unacceptably high. All studies point to the virtual certainty that the "Chehalis problem" is caused by a pathogen. On that basis we recommend that an intensified search be continued for the unknown agent.

RESUME

Alderdice, D. F. and D. R. Harding. (Editors). 1987. Studies to determine the cause of mortality in alevins of chinook salmon (Oncorhynchus tshawytscha) at Chehalis Hatchery, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1590: 96 p.

La piscifacture de Chehalis située sur les rives de la rivière Chehalis, tributaire de la rivière Harrison dans la vallée supérieur du Fraser (Colombie-Britannique), a été mise en service en 1982. Les oeufs de plusieurs espèces de saumons du Pacifique, dont le saumon quinnat, y sont incubés. Depuis l'ouverture de la piscifacture, les alevins quinnats ont fait l'objet d'un taux élevé de mortalité à partir de plusieurs semaines après l'éclosion. Le stock de saumon quinnat provient de la rivière Harrison avoisinante. Ce problème, depuis qu'il a été observé la première fois, fait l'objet d'une étude pathologique soutenue. Étant donné qu'aucun agent pathogène n'a été lié à ce problème, on s'est penché sur d'autres facteurs dont la susceptibilité génétique du stock de la rivière Harrison et d'autres stocks et l'influence de l'approvisionnement en eau plutôt douce de la piscifacture de Chehalis sur la réaction des alevins à l'apport d'ions au milieu d'incubation (Ca^{+2}, Na^{+}, K^{+}). Jusqu'à maintenant, aucun agent pathogène n'a été lié au problème. Par contre, des études ont révélé que l'agent responsable doit être un microbe pathogène. Des études génétiques ont révélé que le stock de la rivière Harrison est particulièrement sensible à ce problème et que cette susceptibilité est en grande partie transmise par les mâles. Il est intéressant de noter que le stock de saumon quinnat de la rivière Harrison se compose en grande partie (environ 98%) de quinnat "à chair blanche". En comparaison, les quinnats "à chair rouge" de la rivière Capilano (antérieurement le stock de la rivière Big Qualicum) montrent une faible susceptibilité à cet agent pathogène. On sait que les salmonidés ont une incidence accrue de problèmes physiologiques et pathologiques dans des eaux douces où les teneurs en Ca^{+2}, Na^{+} ou K^{+} sont faibles. L'apport de ces ions à l'approvisionnement d'eau d'incubation révèle que des teneurs accrues de Ca^{+2} et de Na^{+} réduisent nettement le taux de mortalité entrainé par l'agent pathogène présumé. Néanmoins, les taux de mortalité des saumons quinnats gardés dans des milieux ajoutés de Ca^{+2} et de Na^{+} à la piscifacture de Chéhalis sont toujours trop élevés. Toutes les études indiquent de fait que "le problème de Chehalis" est lié à un agent pathogène. Sur ce fait, nous recommandons la poursuite d'une recherche plus poussée visant à identifier l'agent inconnu.

INTRODUCTION

Since it began operating in 1982, the Chehalis Hatchery, located near Harrison Mills, B.C., has had continuing problems with mortality among recently hatched alevins of chinook salmon (Oncorhynchus tshawytscha) of Harrison River origin. Although other species of salmonids have been exposed to the same incubation conditions, mortality of the nature and extent observed has been confined to the Harrison chinook salmon stock. Initially, there appeared to be some evidence that a pathogen might be involved. Further work supports this view, and Traxler and Rankin give evidence based on symptoms, treatment and tissue analyses (Report I). A genetic component also has been identified by R. E. Withler; this work, to be published elsewhere, is summarized herein (Report II) to complete this review of available information. In addition, characteristics of the Chehalis Hatchery water supply suggested that hydromineral metabolism and ionoregulation might be involved. Accordingly, in 1984, groups of affected and unaffected alevins were analyzed for hydromineral content (Dept. Fish. Oceans, Vancouver, unpubl. data); these data are presented by Alderdice and Rombough (Report III). A further study was undertaken in 1985-86, based on ionoregulatory problems known to occur in teleost eggs and larvae in soft or acidified fresh waters (Alderdice, Jensen, and Velsen, Report IV). Report IV contains an Addendum, summarizing the results of the latest studies on water quality (1986-1987). Samples of eggs and alevins also were obtained in 1985-86 to examine their hydromineral content throughout development (Alderdice and Hally, Report V). A final summary of the Chehalis Hatchery problem is presented by D. R. Harding.

The Chehalis Hatchery is located on the gravel fan of the Chehalis River about 3 km from its junction with the Harrison River. Its groundwater supply is obtained from on-site wells that draw from an aquifer apparently influenced by the Chehalis River drainage. Surface water is obtained from the Chehalis River. There is said to be a remnant population of chinook salmon spawners native to the Chehalis River, but most of the spawning stock used in Chehalis Hatchery is Harrison River stock. From 1981 to 1984, Harrison River chinook eggs were incubated and the fry reared to release at the Chilliwack River Hatchery on another Fraser River tributary nearby. The 1982 to 1984 brood eggs were from egg lots split with the Chehalis Hatchery. At the Chilliwack River Hatchery, these eggs appeared to be free of problems. In some of the investigations reported here, comparisons also were made with chinook stock from Capilano Hatchery, located on the Capilano River, north shore of Burrard Inlet, near the mouth of the Fraser River. Originally there was no native run of chinook salmon to the Capilano River, and a stock was introduced from Big Qualicum Hatchery, located on the Big Qualicum River, on the east side of Vancouver Island.

This movement of native stocks to different locations and water supplies suggests immediately that a comparison be made of the water characteristics of the various sites involved (Table 1). The tabled values are drawn from various Department of Fisheries and Oceans data sources and are representative of the waters in which chinook salmon eggs would incubate. At Capilano Hatchery the river water is very cold during the winter months and

Table 1. Comparison of waters involved in the examination of the Chehalis Hatchery chinook alevin problem. The Chehalis Hatchery water supply (wells) is from an underground reservoir likely maintained by the nearby Chehalis River. Most Chehalis Hatchery broodstock is native to the Harrison River. Some comparisons made in the investigations involve chinook alevins from the Capilano River, and the origin of the Capilano chinook broodstock is the Big Qualicum River, Vancouver Island, B.C. Other comparisons were made at the Chilliwack and Rosewall Hatcheries. (BDL: below detection limits).

Water characteristic	Chehalis Hatchery (wells)	Chehalis River	Harrison River	Chilliwack Hatchery (wells)	Capilano Hatchery* (river)	Capilano Hatchery* (wells)	Big Qualicum Hatchery (river)	Rosewall Hatchery (wells)
pH	6.4- 6.6	6.3- 6.8	7.1	8.0-8.4	6.8	7.1	7.5-7.8	7.2-7.8
Conductivity µmho/cm	27.4-32	16.8-29.4	48.5	163-170	15.1	62.9	72-86.5	63.5-76.4
Hardness mg/L as CaCO3	9.5-11.2	7.3- 9.5	18.7	53.4-85.2	5.7	20.9	32.9-42.8	30-36
Al mg/L	0.004-0.008	-	0.07	BDL	0.12	BDL	<0.05	-
Fe mg/L	0.007-0.136	0.01-0.328	0.07	0.015-0.056	0.18	BDL	0.03-0.065	0.02-0.28
Ca mg/L	3.3-3.5	2.4-4.0	6.1	20-27	1.5	5.2	11-14.1	9.9-10.8
Na mg/L	0.8-0.9	0.26-0.73	1.2	6.6-7.2	0.6	2.8	0.98-1.84	0.4-3.1
K mg/L	0.07-0.126	0.12-0.16	0.54	0.6-1.9	0.24	1.41	0.13-0.21	-
Mg mg/L	0.3-0.4	0.27-0.36	0.7	1.3-1.8	0.20	1.9	1.32-2.26	1.2-2.6
Cu mg/L	0.0016	BDL	<0.001	BDL	BDL	BDL	BDL	-
Zn mg/L	0.0054	<0.0042	<0.002	<0.02	BDL	BDL	BDL	-

*Capilano Hatchery uses river and well water mixed or unmixed according to temperature; see text.

growth and development would be very slow in river water in that period. Well water at Capilano Hatchery is from the same watershed, is modified during underground percolation, and has a constant temperature of 7.0-7.1°C. In practice, egg incubation starts in the higher temperature river water, is switched to well water when river temperatures decline to that of the well water, and later developmental stages are returned to river water with the spring rise in temperature. Indeed, the major reason for using well water at Capilano Hatchery is the fact that its temperature is higher and more constant during the winter months, in comparisons with surface (river) runoff.

At Chehalis Hatchery, groundwater temperatures are near 10°C at the time of first egg collections. Temperatures drop gradually to a minimum of about 4.5 °C in early March and begin their annual upward trend in late March. The progress of egg and alevin development is related to "accumulated temperature units" (ATU). That is, the average daily water temperature during incubation in degrees Celcius (above zero °C) is accumulated daily. All chinooks eggs were treated with malachite green three times weekly until the eyed embryo stage (approx. 300 ATU), then discontinued. Ovadine treatment, tried in previous years, had no beneficial effect on the unknown problem affecting the alevins. It was discontinued except in two instances (Reports I, II) where it was continued to duplicate conditions in previous years. All eggs were shocked and picked at approximately 300 ATU to eliminate dead and infertile eggs.

The last section of this report (Summary) attempts to relate the various findings of the Investigational Reports in an effort to interpret the alevin mortality problem.

ACKNOWLEDGMENTS

Acknowledgments of the authors' papers are consolidated herein. The various projects were initiated by G. S. Traxler, R. E. Withler, P. J. Rombough and D. F. Alderdice, and coordinated by D. R. Harding. They express their appreciation to L. Kahl, Project Manager, Chehalis Hatchery, and staff, for facilitating the studies. Chinook salmon gametes (genetics project) were supplied by Capilano and Chehalis Hatchery managers, E. Stone and L. Kahl, and able assistance was provided by D. Tuck, R. Stitt, and N. Gayou. In particular, we thank K. Horobin who sampled alevins, recharged stock solution containers (water quality projects), recorded experimental results, and in general controlled all projects. The editors also thank Drs. L. Margolis, T. Evelyn and E. Donaldson, and L. Kahl and K. Horobin for reviewing the various manuscripts.

Investigational Reports

Pathology (Report I)

I. Traxler, G. S. and J. B. Rankin. 1987. Pathological aspects of the Chehalis Hatchery chinook alevin problem. In: D. F. Alderdice and D. R. Harding [Eds.]. Studies to determine the cause of mortality in alevins of chinook salmon (Oncorhynchus tshawytscha) at Chehalis Hatchery, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. No. 1590: 5-17.

INTRODUCTION

The Chehalis River Hatchery, located on the lower Chehalis River near the confluence with the Harrison River, began operations in 1982. Using standard fish culture procedures the hatchery raises the following species: Oncorhynchus keta, O. tshawytscha, O. kisutch (chum, chinook, and coho salmon) and Salmo gairdneri, S. clarki (steelhead and cutthroat trout). The Harrison River has been the source of chinook broodstock for both the Chehalis Hatchery and the Chilliwack Hatchery.

In each year of operation excessive chinook alevin losses have occurred at the Chehalis Hatchery. The losses have ranged from a low of 21% in the 1982 brood year to a high of 81% in the 1984 brood year (Table 1). The majority of the mortalities occur between 650-750 accumulated temperature units (ATU), but can carry on to the 870-880 ATU stage. Mortalities can begin in late December and continue until mid-February, depending upon the date the eggs were fertilized and the water temperature during incubation. Species other than chinook salmon raised at Chehalis have not suffered excessively high losses. The same Harrison chinook stock raised at the Chilliwack Hatchery has had low alevin mortalities and no indication of the same problem.

Table 1. Annual chinook alevin mortalities at the Chehalis River Hatchery.

Brood year	Mortality (%)
1982	21
1983	24
1984	81
1985	45

Eggs in vertical stacks of incubation (Heath) trays initially appear to be affected randomly. However, once a tray is affected, trays below it usually show losses several days later. In some cases the problem may skip exposed trays but this is a very rare occurrence. In the 1983 brood year two complete head tanks and the incubation stacks they supplied (approx. 1 million eggs) were unaffected; the eggs in this group were taken from the middle portion of the chinook run.

Prior to the onset of mortality alevins become lethargic. When trays are pulled from a stack, affected alevins show little reaction to light or disturbance. When handled in the same manner unaffected trays of alevins set up a strong circular swimming pattern. Affected alevins also are paler than non-affected alevins. When examined under low power (60X), blood flow in the yolksac vessels of affected alevins is congested; in spite of a strong and regular heart beat, the blood flow through the yolksac capillaries either is very slow or oscillates, acting as though the blood were showing a loss of plasma and increased viscosity, or that some blood vessels were constricted. Unaffected alevins do not show signs of congested blood flow. Mortality in affected trays can vary from 10% to 100%. As mortalities accumulate a mat of fungus and bacteria forms that tends to trap the alevins remaining in the tray.

In the 1982 and 1983 brood years routine diagnostic procedures were conducted in an attempt to identify the cause of the losses. In the 1984 brood year extensive efforts were undertaken to identify a virus as the cause of mortality, as no parasitic or bacterial agent was identified in the two previous years. Prior to the 1985 egg take, three experiments were designed to determine whether an infectious agent was the cause of the problem. This report is a review of the work done in each of the years to determine if a pathogen is responsible for the losses.

RESULTS

1982 BROOD YEAR

When losses of chinook alevins occurred in the 1982 brood year, the first mortalities were reported and examined on January 18, 1983. The fish were sluggish and there were many moribund alevins in the trays. Wet mounts of the gills revealed large amounts of fungus as well as a variety of bacteria. No virus was isolated in a virus assay of the affected yolksac fry. Alevins submitted for histology revealed gill hyperplasia and hypertrophy. Bacteria and fungus also were present in gill sections from some fish, likely those sampled from the center of affected trays.

1983 BROOD YEAR

During the 1983 brood year the diagnostic service from the Pacific Biological Station sampled the chinook fry for infectious disease agents three times during the period in which losses occurred. The affected fry showed a marked reduction in activity when disturbed. There was an accumulation of precipitated protein from ruptured yolk sacs of dead fish. Grossly, the fry appeared normal, with no deformities nor irregularities in the yolk sac.

Microscopic examination of the gills by wet mount showed some invasion by fungus as well as a clogging of the filaments with debris.

Histological sections of affected fry, both moribund and freshly dead, indicated all internal organs were normal. No necrosis suggestive of an infectious agent was observed internally. Sections of the gills showed moderate to heavy hyperplasia. Fungus was also present in some of the affected fry. Inability to identify a parasitic or bacterial pathogen as the cause of the mortalities leads to the suggestion that a viral pathogen may have been responsible. The species and age of the fish suffering mortality would indicate that infectious hematopoietic necrosis virus (IHNV) may have been the causative agent.

The first sample for virology was collected in early January, 1984, shortly after losses had started. Fifty affected alevins were processed in a routine manner in pooled samples of five fish each. Three fish cell lines, rainbow trout gonad (RTG-2), fathead minnow (FHM), and epithelioma papillosum cyprini (EPC) were inoculated in duplicate and incubated at 10 and 15°C. During 14 days of incubation no evidence of a viral agent was observed.

The next sample, collected for virological tests in mid-January consisted of 60 moribund and 60 freshly dead alevins. The fish were pooled in groups of 5 and homogenized after the removal of the yolk sac. Samples were inoculated on three cell lines; RTG-2, FHM, and Chinook salmon embryo (CHSE). After 14 days of incubation at 15°C a blind passage, to enhance virus isolation, was done on all samples. A further 14 days of incubation did not result in the isolation of virus.

The third set of virology samples was obtained in early February when losses were nearing an end. Sixty moribund fish were pooled in groups of 5 and homogenized without the removal of the yolk sac. FHM and RTG-2 cell lines were inoculated and incubated at 15°C for 14 days. No evidence of virus was observed in any of the cell cultures. The procedures followed for virology were extensive and would have resulted in the isolation of any known salmonid viruses that could have been grown in cell culture.

Wet mounts and histological sections of gills again showed extensive gill hyperplasia. Fungus also was present in many of the affected sac fry. It is unusual for a fungal infection of sac fry to be limited to the gills; as well, the appearance of fungus in the affected trays usually occurred after losses had begun. This would indicate that the fungus was a secondary problem and that some other debilitating factor was the primary cause. We surmised that an unrecognizable pathogen could be involved, one that caused very little tissue damage and one not amenable to laboratory culture using conventional techniques.

1984 BROOD YEAR

In the 1984 brood year losses of chinook alevins reached 81%. As a result of these excessive losses, an intensive effort to identify an infectious agent was undertaken. Again, most of the effort was concentrated on attempting to isolate or identify a viral pathogen. In the 1984 brood year one hatchery procedure was changed. Eggs from female chinook were not pooled and each Heath tray contained eggs from only one female.

Virology. Initially, virus assays consisted of two cell lines, FHM and RTG-2, and these were incubated at 15°C for two weeks (Can. Dept. Fish. Oceans 1984). No virus was isolated using these procedures. To increase the chances of isolating a virus the number of cell lines used was increased to five by adding the following cell lines: EPC, sockeye salmon embryo (SSE-5), and chinook salmon embryo (CHSE-214). Duplicate samples were incubated at 4 and 15°C. After two weeks of incubation a blind passage was done on all samples, which were examined microscopically over a two week period. Freshly dead and moribund alevins collected several times during the period of heavy mortality were assayed using these procedures; the tests failed in the isolation of any virus.

Assistance in testing for virus in the dying chinook sac fry was requested from two fish health laboratories in the United States. Typically affected chinook alevins were sent alive on ice to the National Fishery Research Center in Seattle, and the Marine Science Center of Oregon State University. Fish health experts from both laboratories were unable to isolate a virus from the samples.

Hematology. The flow of blood was markedly reduced in affected fry. In spite of a strong regular heart beat the blood flow was very slow or oscillated in the capillaries of the yolk sac. In contrast, in unaffected groups the blood flowed rapidly through the capillaries.

Blood smears were prepared, stained with *Diff-Quik stain, and examined microscopically for any abnormalities. Smears were prepared from 25 different groups of chinook alevins that had suffered mortality ranging from 0-90%. Control groups of Big Qualicum and Chehalis chinook being reared at Rosewall Creek on Vancouver Island also were examined. Blood smears from the affected groups generally showed the following characteristics when compared to normal chinook alevins.

1. There was an increased number of white blood cells in the peripheral blood. These included numerous macrophages, which were often vacuolated. Circulating white blood cells were observed far less frequently in the control groups.

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*Dade Diagnostics, Inc. Aguada, Puerto Rico 00602

2. Blood smears from the affected alevins had a higher proportion of mature vs. immature erythrocytes than did blood from the non-affected alevins. It appeared that the hematopoietic tissues of affected fish were not producing new erythrocytes at the rate required for development of rapidly growing alevins.

3. Affected alevins had a high proportion of erythrocytes without a nucleus. Many of the erythrocytes had eccentric nuclei. In some affected groups there was a number of vacuolated erythrocytes.

Histology. Histological sections of affected alevins revealed much the same evidence as seen in previous years. Affected groups showed normal internal organs, with only the gills having consistent abnormal pathology. Each affected group showed extensive gill hyperplasia with some hypertrophy.

Electron Microscopy. The use of the electron microscope to diagnose viral diseases is not a sensitive method. This is especially true if the size, shape and etiology of the pathogen is unknown. Several attempts to detect a pathogen using the electron microscope were made, as none of the previous assay methods used led to the isolation of the causative agent. Normal and affected alevins were fixed with 4% gluteraldehyde in Millonigs buffer. The samples were post-fixed with osmium tetroxide, sectioned and stained, and examined by electron microscopy. Several tissues were examined including the gills, kidney, brain, heart, and digestive tract. A few erythrocytes were observed, which contained virus-like particles similar in shape to some of the erythrocytic necrosis viruses (ENV). The particles, however, were small (20 nm) relative to the known EN viruses (Rohavec and Amandi 1981). Because few of these particles were observed, it is unlikely these particles were responsible for the mortality occurring.

Hatchery Experiments. A few experiments were conducted by inserting unaffected trays of alevins under affected trays during the 1984 brood year.

The susceptibility of Capilano chinook was tested by placing two trays of alevins under trays of affected Harrison chinook alevins. After 13 days of exposure the Capilano chinook remained active with no losses.

Harrison chum alevins were tested for susceptibility by placing a control tray of chum alevins at the top of a stack and duplicate trays of chum alevin under 7 affected trays of chinook alevins. The tray of chums exposed to affected chinook suffered a 60% loss after 7 days, and losses continued to 95% in 15 days. No losses occurred in the tray of chums placed at the top of a stack.

Harrison chinook alevins incubated and hatched at the Chilliwack hatchery also were susceptible when returned to Chehalis Hatchery and challenged as alevins. After 8 days of exposure to affected fish, losses ranged from 0-30% depending upon the location in the stack (see Table 2). Mortalities continued and finally reached 90% in 14 days. The incubation period between the initial exposure and the onset of mortalities was indicative of a pathogenic agent.

Table 2. Mortality patterns among Harrison chinook alevins incubated and hatched at the Chilliwack Hatchery (Trays 2, 4, 6, 8) when returned to Chehalis Hatchery and challenged by exposure to affected alevins hatched at Chehalis Hatchery (Trays 1, 3, 5, 7).

Tray	Jan. 23-85	Jan. 31-85	Feb. 5-85
1	Harrison chinook 90% mortality		
2	Chilliwack chinook	0% mortality	60% mortality
3	Harrison chinook 90% mortality		
4	Chilliwack chinook	10% mortality	70% mortality
5	Harrison chinook 80% mortality		
6	Chilliwack chinook	20% mortality	70% mortality
7	Harrison chinook 80% mortality		
8	Chilliwack chinook	30% mortality	90% mortality

1985 BROOD YEAR

Prior to the 1985 egg take several experiments were designed to determine if the chinook alevin losses were being caused by an infectious agent. Except for the eggs taken on October 31, 1985 the adult Harrison chinook were checked for bacterial and viral pathogens that could have influenced the survival of the progeny. No bacterial or viral pathogens were isolated from the adult chinook.

Experiment A. This experiment was designed to determine the susceptibility of chum and non-indigenous chinook stocks (Table 3). Harrison chinook eggs from the October 31 egg take were pooled and divided equally among 4 Heath trays. Eggs from the November 6 spawning were pooled similarly and divided into 8 trays. Eyed chinook eggs from the Chilliwack and Big Qualicum hatchery were pooled separately and each group was divided and placed into 4 trays. Eyed chum eggs from the Chehalis river were pooled and divided into 4 replicate trays. The various trays of eggs then were placed in the incubator stacks and monitored (Table 3).

Results

1. First losses occurred among the Harrison chinook alevins in tray 7G5 on January 27. Losses occurred sequentially in trays below tray 7G5. This pattern -- of fish becoming affected several days after exposure to fish in trays above -- is suggestive of an infectious agent.

2. Only 1 of 4 trays of Harrison chinook alevins from the October 31 eggs was affected; this was the only tray challenged by exposure to affected alevins located in a tray above.

3. Only 6 of the 8 trays of Harrison chinook spawned on November 6 were affected, even though the eggs in all trays were pooled from the same brood stock.

4. Only the Big Qualicum chinook that were challenged showed losses and these losses were much lower than those in any of the trays of challenged Harrison chinook alevins.

5. Only the Chilliwack chinook that were challenged showed losses.

6. Once mortalities began in a tray the majority of losses occurred within one week.

7. Chehalis chum showed no susceptibility.

Experiment B. The objective of this experiment was to determine if the causative agent is on the surface or inside of the egg. Four horizontal rows of trays were loaded with pooled eggs from 4 Harrison female chinook (Table 4). The lower 2 trays in each stack contained pooled Chehalis chum eggs. The well water flowing into stacks 5I and 5J was treated with ultraviolet light (21,370 microwatt seconds/cm^2). Trays in stacks 5K and 5L received

Table 3. Configuration of Heath trays in experiment A. Below each tray of eggs is shown the date of the egg take (when known), the date when losses began, and the cumulative percent mortality in that tray until ponding. The fry from the October 31 egg take were ponded on March 1, all other trays were ponded on March 13.

Tray	Stack 7A	7B	7G	7H
2	Harrison chinook Oct. 31/ - / 0%	Harrison chinook Oct. 31/ - / 0%	Chilliwack chinook - / - / 0%	Chilliwack chinook - / - / 0%
3	Harrison chinook Nov. 6/Feb. 3/98%	Harrison chinook Nov. 6/Feb. 19/92%	Big Qualicum chinook - / - / 0%	Big Qualicum chinook - / - / 0%
4	Harrison chinook Nov. 6/Feb. 10/94%	Harrison chinook Nov. 6/Feb. 19/88%	Chehalis chum - / - / 0%	Chehalis chum - / - / 0%
5	Chilliwack chinook - /Feb. 12/98%	Chilliwack chinook - /Feb. 21/75%	Harrison chinook Nov. 6/Jan. 27/98%	Harrison chinook Nov. 6/ - / 0%
6	Big Qualicum chinook - /Feb. 14/45%	Big Qualicum chinook - /Mar. 3/12%	Harrison chinook Nov. 6/Jan. 27/98%	Harrison chinook Nov. 6/ - / 0%
7	Chehalis chum - / - / 0%	Chehalis chum - / - / 0%	Harrison chinook Oct. 31/Feb. 4/83%	Harrison chinook Oct. 31/ - / 0%

Table 4. The arrangement of Heath trays in experiment B. The date below each tray shows when mortality began; the accompanying figure is the final cumulative percentage mortality for each tray. Symbols: WW-well (ground) water; UVT-ultraviolet treatment; SD-surface disinfection.

Tray	Stack treatment	5I WW-UVT SD	5J WW-UVT No SD	5K WW-No UVT SD	5L WW-No UVT No SD
2		Harrison chinook - /0%	Harrison chinook Feb. 10/100%	Harrison chinook - /0%	Harrison chinook - /0%
3		Harrison chinook Feb. 20/100%	Harrison chinook Feb. 12/100%	Harrison chinook Feb. 19/100%	Harrison chinook - /0%
4		Harrison chinook Jan. 30/100%	Harrison chinook Feb. 10/100%	Harrison chinook Feb. 21/100%	Harrison chinook - /0%
5		Harrison chinook Feb. 4/100%	Harrison chinook Feb. 16/100%	Harrison chinook Feb. 25/100%	Harrison chinook - /0%
6		Chehalis chum - /0%	Chehalis chum - /0%	Chehalis chum - /0%	Chehalis chum - /0%
7		Chehalis chum - /0%	Chehalis chum - /0%	Chehalis chum - /0%	Chehalis chum - /0%

untreated well water. The eggs in stacks 5I and 5K were surface disinfected with 100 ppm ovadine for 10 minutes while the eggs in stacks 5J and 5L were not surface disinfected.

Results

1. The first tray affected (5I4) was located in the stack containing surface disinfected eggs and ultraviolet-treated water. Five days later the tray below started showing losses.

2. None of the trays in stack 5L, which received untreated well water, were affected. In stacks 5I and 5J, 7 of the 8 trays receiving ultraviolet treated water were affected. This pattern would indicate the "causative agent" is not in the water supply.

3. Surface disinfection of the eggs did not prevent losses from occurring in the alevins. This would suggest the "causative agent" is either inside the egg or not susceptible to normal surface disinfection procedures.

4. Replicate trays in each horizontal row did not show identical results. In some rows only 1 of 4 replicate trays of alevins were affected. This suggests that the problem of alevin losses is associated with a very low number of eggs in each female that carry the pathogen.

5. Chehalis chum in the bottom two rows showed no susceptibility.

Experiment C The objective of this experiment was to determine whether losses of challenged chinook alevins could be prevented by ultraviolet treatment of effluent water from trays of affected alevins (Table 5). The three upper stacks (5A, 5B, and 5C) were loaded with susceptible Harrison chinook eggs to provide a challenge for the two stacks below (6A and 6C). Eggs in the upper stacks were pooled from 3 females and divided into equal portions by weight, so that trays in each horizontal row contained eggs as identical as possible. The lower two stacks, 6A and 6C, were loaded with susceptible Harrison chinook eggs except that the eggs from two females were pooled. Trays 6 and 7 (stacks 6A and 6C) were loaded with pooled chum eggs. Effluent from the upper stacks (5A, 5B, and 5C) was collected and split. One half was left untreated (6C); the other half passed through a 50 µm filter and ultraviolet lamps (21,370 microwatt sec/cm^2) before passing through stack 6A.

Results.

1. All susceptible Harrison chinook alevins receiving untreated effluent from affected trays located above them suffered high losses.

2. Alevins in tray 6A5 receiving ultraviolet treated water became lethargic on February 4 but no losses occurred and they seemed to recover. Mortalities finally started on February 26 and amounted to 38% prior to ponding. The initial onset of signs as early as February 4 suggests that the cause of the losses came from the eggs initally placed in the tray, rather than from affected alevins located in the trays above. The replicate tray 6C5 also began showing losses on February 4.

Table 5. Experiment C - arrangement of trays. Stacks 5A, 5B, and 5C received unfiltered, untreated (no UV treatment) water. The water exiting the three upper stacks was gathered and divided equally between the lower stacks. That going to stack 6A was filtered and UV treated; that going to stack 6C was unfiltered and untreated. For each tray the table shows the species and source. The date when mortality started and the final cumulative percentage mortality until ponding is shown below each tray. No losses occurred in stacks 5A, 5B, 5C, trays 2, 3 or 4, nor in the chum trays.

Tray	5A	5B	5C
2	Harrison chinook	Harrison chinook	Harrison chinook
3	Harrison chinook	Harrison chinook	Harrison chinook
4	Harrison chinook	Harrison chinook	Harrison chinook
5	Harrison chinook Jan. 28/100%	Harrison chinook Feb. 10/100%	Harrison chinook - /0%
6	Harrison chinook Feb. 6/100%	Harrison chinook Feb. 10/100%	Harrison chinook - /0%
7	Harrison chinook Feb. 10/100%	Harrison chinook Feb. 10/100%	Harrison chinook Mar. 7/100%

	UV treated-filtered	untreated
	6A	6C
2	Harrison chinook - /0%	Harrison chinook Feb. 10/100%
3	Harrison chinook - /0%	Harrison chinook Feb. 10/100%
4	Harrison chinook - /0%	Harrison chinook Feb. 10/72%
5	Harrison chinook Feb. 4/38%	Harrison chinook Feb. 4/100%
6	Chehalis chum	Chehalis chum
7	Chehalis chum	Chehalis chum

3. The only susceptible alevins below affected fish that did not suffer losses were the 3 trays of Harrison chinook directly below the filter and ultraviolet treatment (6A, trays 2, 3, 4).

4. After stack 6C had suffered complete losses, about 100 fry from 6A2 and 6A3 were moved to 6C2 and 6C3, respectively. An affected tray of fry was placed above in location 5B7. After 17 days losses began and finally reached 30%.

SUMMARY

In each year of operation the Chehalis River hatchery has suffered significant losses of the Harrison River chinook alevins. Losses occur during the period of yolk absorption. The alevins first become lethargic and do not react to light or physical disturbance. Blood flow in affected fish is congested. There is a strong regular heartbeat but the blood flow is almost static in the capillaries of the yolk sac.

The only tissues that appear to be affected are the blood and the gills. Internal organs and tissues of affected fish appear normal.

Blood smears from affected groups of alevins show the following differences from those of non-affected groups.

1. A high proportion of erythrocytes lacking a nucleus.
2. Many erythrocytes have an eccentric nucleus.
3. Vacuolation in the cytoplasm of the immature erythrocytes.
4. Numerous white blood cells in the circulating blood.
5. A higher proportion of mature vs. immature erythrocytes in the blood of affected chinook alevins.

The gills of affected fish are also abnormal. There is extensive hyperplasia of the epithelium. Gas exchange is probably greatly reduced as a result of the thick layer of epithelial cells. The congested blood flow observed in the alevins may be due to the extreme gill hyperplasia.

Hyperplasia of the gills can be caused by a variety of chemical and physical irritants as well as bacteria (Eller 1975). Bacterial gill disease is usually associated with poor environmental conditions. The gills in none of the affected fry showed the characteristic long thin myxobacteria which are typical of bacterial gill disease.

In spite of the evidence suggesting that an infectious agent is involved in the losses, none has yet been isolated. Standard bacteriological and extensive virological tests have failed to identify or isolate a pathogen.

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INTRODUCTION

At Chehalis Hatchery, the rate of chinook salmon (Oncorhynchus tshawytscha) alevin mortality varies among years, incubation stacks, trays within incubation stacks and, apparently, among stocks. For salmonids, genetic variation has been documented within and among populations for survival under adverse environmental conditions and in the presence of disease or parasitic organisms (Gjedrem and Aulstad 1974; Amend and Nelson 1977; Ehlinger 1977; Ihssen 1978; Edwards and Gjedrem 1979; Winter et al. 1980; Hemmingsen et al. 1986). In this study, the possibility is investigated that genetic variation contributes to the variable survival rates of chinook salmon alevins at Chehalis Hatchery.

Harrison River chinook salmon are predominantly white-fleshed fish (Godfrey 1975) that produce pale orange eggs with a low carotenoid pigment content. Survival of the carotenoid-rich deep orange eggs of red-fleshed chinook salmon stocks may be better than that of the paler eggs of white-fleshed fish under Chehalis Hatchery incubation conditions (Larry Kahl, Chehalis Hatchery manager, pers. comm.). In this study, crosses were made among and within white-fleshed Harrison River and red-fleshed Capilano River chinook salmon adults to measure genetic variation in resistance to (tolerance of) the mortality-inducing agents at Chehalis River Hatchery.

MATERIALS AND METHODS

Gametes from Capilano River (originally Big Qualicum River stock) and Harrison River chinook salmon adults were used to make two sets of factorial crosses at Chehalis Hatchery. In each set, four Harrison and three Capilano females were crossed with three Harrison and three Capilano males to produce a total of 42 families, which included pure Harrison, pure Capilano and the reciprocal hybrid families. Two replicates of each family were made, and the 84 replicates of each set were incubated in small baskets fitted into five trays of adjacent incubation stacks with the same water source.

Twenty-five days after hatching, the two sets of five incubation trays were each placed beneath a single tray of affected Harrison River chinook alevins, among which mortality was occurring. Mortality among the

experimental alevins began four days after exposure to the affected alevins. Dead alevins were counted and removed three to six times weekly for a 49-day period, by which time mortality had ceased almost completely.

A mixed model analysis of variance was used to determine the significant sources of variation in survival (treated as a binomial trait; dead = 0, live = 1) and time to death (measured in days after exposure) for the purebred alevins (Harrison x Harrison, Capilano x Capilano) of each set. The effect of stock (Harrison or Capilano) was considered fixed. Random effects were male parents (sires), female parents (dams), the male-female interaction, and replicates, all nested within stocks. Sire and dam heritability estimates and their standard errors for proportionate family survival and time to death were calculated independently for purebred Harrison and Capilano alevins according to Becker (1984) and Bogyo and Becker (1965). For each stock, a mixed ANOVA model was used in which the random effects of sires, dams, and the sire-dam interaction were all nested within the fixed effect of stacks.

RESULTS

SURVIVAL

Survival to the eyed egg stage was greater than 50% for all crosses except those involving a single female of Set 1, for which survivals were less than 1%. The six families involving this female were excluded from the study, and Set 1 was reduced to a cross of six females by six males. For the remaining families, mean (±S.E.) survival to the eyed stage was 89.1 ± 1.08%, and, after hatching, the number of live alevins per replicate ranged from 75 to 325.

The experimental alevins of both Sets 1 and 2 rapidly developed typical symptoms of the affliction (listlessness, altered skin chromatophore response) upon being placed beneath trays of affected alevins, and mortality began on the fourth day after exposure. By the 49th day after exposure, mortality had decreased to very low levels, and the surviving alevins were considered to have resisted or tolerated the mortality-inducing factors.

Alevin survival by family (averaged over the two replicates) ranged from 0 to 100%. Mean survival in Set 1 was 27.9%, in Set 2 it was 41.4%, and over all families it was 35.2%. In purebred crosses, mean survival for Harrison families was 13.0%, and for Capilano families, 64.1% (Table 1). The difference in survival between stocks was significant in Set 1 ($F_{1,6} = 10.0$, $P<0.05$) and approached significance in Set 2 ($F_{1,7} = 3.5$, P 0.1). In both sets, survival varied significantly among male ($F_{4,8} = 9.4$, $P<0.01$; $F_{4,10} = 10.3$, $P<0.01$) and female ($F_{4,8} = 5.0$, $P<0.05$; $F_{5,10} = 6.5$, $P<0.01$) parents within stocks. There was no significant male-female interaction in either set ($F_{8,18} = 2.3$, $P>0.05$; $F_{10,21} = 1.9$,

P>0.05), but in both sets there was significant heterogeneity in survival between family replicates ($F_{18,5766}$ = 21.0, P<0.01; $F_{21,6219}$ = 27.3, P<0.01).

Survival in the interstock crosses generally was intermediate to purebred survival levels, but more closely resembled survival of the paternal stock than of the maternal stock (Table 1; Fig. 1). In Set 1, in which mortality was greatest, survival of the Harrison male x Capilano female crosses deviated 92% from the mid-stock value towards the survival value of pure Harrison crosses. Survival of hybrid crosses with Capilano male parents deviated from the mid-stock value 47% towards the value of pure Capilano crosses.

In Set 2, mortality occurred more slowly, and reached a lower overall level in the pure Harrison crosses (Table 1). Survival levels of the interstock hybrids in Set 2 were closer than those of Set 1 to mid-stock values (Table 1; Fig. 1). The mean survival of hybrid families with both Harrison and Capilano fathers deviated slightly (13% and 7%, respectively) from the mid-stock value towards the survival level of pure Harrison families.

For survival of purebred Harrison families, the sire component estimate of heritability was high (0.88 ± 0.52) whereas the dam component estimate was low (0.03 ± 0.04). In purebred Capilano families, both the sire and dam component heritability estimates were high (0.81 ± 0.53 and 0.76 ± 0.50, respectively).

TIME TO DEATH

For those alevins that died, the average time to death by family ranged from 7.5 to 48.0 days after exposure (Table 1). The mean time to death in Set 1 was 14.2 days and in Set 2 was 23.5 days. The mean time to death for pure Harrison families was 15.3 days and for pure Capilano families was 21.8 days (Table 1). The difference between stocks in time to death was significant in Set 1 ($F_{1,5}$ = 6.5, P = 0.05), but not in Set 2 ($F_{1,7}$ = 1.0, P>0.05). There was significant variability among male and female parents in Set 2 ($F_{4,9}$ = 12.6, P<0.01; $F_{5,9}$ = 5.8, P<0.05), but not in Set 1 ($F_{4,8}$ = 2.4, P>0.1; $F_{4,8}$ = 2.9, P = 0.1). There was no significant interaction between male and female parents in either set ($F_{8,18}$ = 0.8, P>0.05; $F_{9,20}$ = 0.9, P>0.05), but time to death was significantly heterogeneous between family replicates in both sets ($F_{18,3790}$ = 15.7, P<0.01; $F_{21,6219}$ = 26.7, P<0.01).

Among interstock hybrids, the time to death more closely resembled that of the paternal stock than that of the maternal stock (Table 1). For hybrids with Capilano fathers, time to death deviated from the mid-stock value towards the pure Capilano value 79% in Set 1 and 129% in Set 2. For the hybrid alevins sired by Harrison males, time to death deviated from the mid-stock value towards the pure Harrison value 47.8% in Set 1 and 29.4% in Set 2.

Table 1. Mean family survival rate and time to death (days) for purebred and hybrid Harrison and Capilano chinook salmon alevins at Chehalis Hatchery.

		Survival rate			Time to Death		
Male	Female	Overall	Set 1	Set 2	Overall	Set 1	Set 2
Harrison x	Harrison	0.130	0.001	0.226	15.28	11.00	18.49
Harrison x	Capilano	0.219	0.025	0.412	17.05	12.54	21.56
Mid-Parent		0.386	0.314	0.441	18.52	13.95	22.84
Capilano x	Harrison	0.441	0.462	0.425	23.23	16.29	28.43
Capilano x	Capilano	0.641	0.628	0.655	21.75	16.90	27.19
Overall		0.352	0.279	0.414	19.10	14.19	23.47

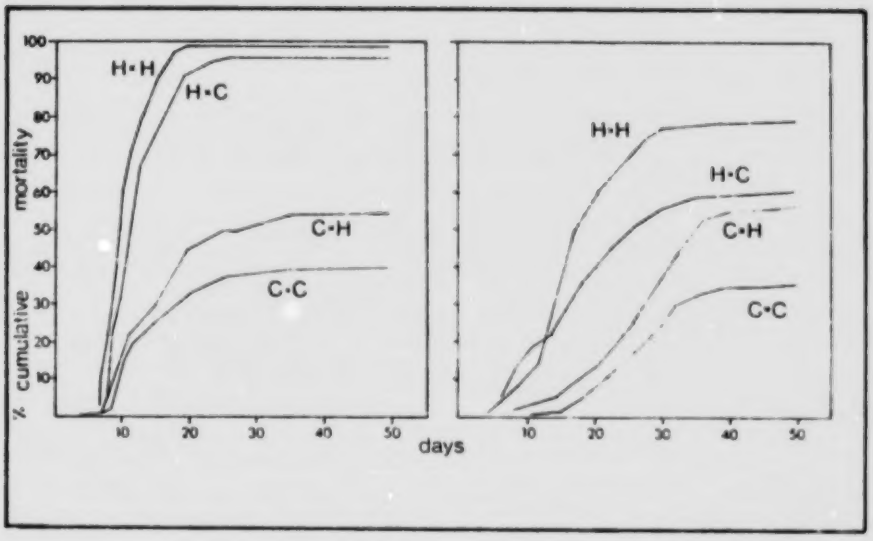

Fig. 1. Cumulative mortality (percent) over time (days after transfer) for chinook salmon alevins of Sets 1 and 2. All families are pooled for each type of cross: H x H (Harrison x Harrison); H x C (Harrison male x Capilano female); C x C (Capilano x Capilano); C x H (Capilano male x Harrison female).

Sire and dam component estimates of the heritability of time to death were high for the Harrison stock (1.39 ± 0.87 and 0.71 ± 0.46), but low for the Capilano stock (0.06 ± 0.11 and 0.17 ± 0.18). The sire component estimates of the genetic correlation between survival and time to death were 1.0 among Harrison families and 0.0 among Capilano families.

DISCUSSION

Mortality of the Harrison and Capilano stock chinook salmon alevins incubated at Chehalis Hatchery occurred after the alevins were "challenged" by exposure to affected alevins. Death may have been the result of poor water quality in the affected incubation stacks, of a pathogen within the experimental groups or transmitted to them by water flow from affected alevins, or of a combination of factors (Alderdice and Rombough 1987; Alderdice et al. 1987; Traxler and Rankin 1987). As predicted, Capilano alevins survived better than Harrison alevins in both sets of crosses.

This study revealed that resistance (tolerance) to the mortality-inducing factors is under strong genetic control in chinook salmon alevins. Contrary to expectation, survival was not a maternally controlled function of egg carotenoid levels. Instead, there was a very strong paternal influence on survival and time to death. For pure Harrison alevins, the high male component and low female component heritabilities of survival indicated that the level of survival was determined almost entirely by the male parent. For pure Capilano alevins, the genetic influences of both male and female parents were large and approximately equal. For the hybrid alevins of Set 1, the paternal influence on survival and time to death predominated, whereas for Set 2 hybrid alevins, survival tended to be intermediate to parental values. Thus, for the Set 2 hybrids, both male and female parents determined survival levels, although time to death remained under strong paternal control.

These results indicate that in chinook salmon alevins there may be differential activation of the paternally- and maternally-derived genes that affect survival at Chehalis Hatchery. If the alevins are challenged relatively early in development, only the paternally-derived genetic information is involved in the determination of survival levels and time to death. If the challenge to the alevins is lessened or delayed (e.g., perhaps the Set 2 hybrid alevins), or if the paternal genes confer sufficient resistance to prolong survival (e.g., pure Capilano alevins), then the maternally-derived genes are also expressed and do influence survival. Differential parental gene activation in salmonids has been reported previously (Hitzeroth et al. 1968; Goldberg et al. 1969; Yamauchi and Goldberg 1974; Ihssen 1978).

The mortality of Harrison River chinook salmon alevins reared at Chehalis Hatchery is under genetic influence but does not result from a "genetic defect" in the stock. Harrison River alevins incubated in research hatcheries consistently have survived well (C. B. Murray and R. E. Withler,

unpubl. data). In addition, alevins from other chinook salmon stocks have proven susceptible to mortality at Chehalis Hatchery when exposed to affected Harrison River alevins.

Genetic selection could be practised to produce Harrison River chinook salmon that are more resistant to the mortality-inducing factors by using as broodstock only those adults (especially males) that were themselves incubated at Chehalis. However this selection would be successful only to the extent that each generation of hatchery-incubated alevins were exposed to the mortality-inducing factors, and that the genes that confer resistance do not also result in less desirable changes in traits such as growth or ocean survival.

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INTRODUCTION

The Chehalis Hatchery obtains its incubation water supply mainly from wells and its brood stock from the Harrison River. Since the hatchery became operational, mortality among newly hatched chinook salmon alevins has ranged from about 21 to 81% (Traxler and Rankin 1987). Investigations suggest a pathogen is involved, and susceptibility to the as yet unknown problem is seen to have a genetic component. Yet the problem does not express itself with the same severity when eyed eggs, transferred from the Chehalis Hatchery (Harrison River stock), are hatched and reared at the Chilliwack Hatchery.

About 65-70% of the Na^+, 40-45% of the K^+ and 75-85% of the Ca^{2+} requirements of salmonid alevins, prior to exogenous feeding, are taken up directly from the perfusing water (Rombough and Garside 1984; Peterson and Martin-Robichaud 1986). Sodium is known to be taken up from the external medium from the eyed stage onward in Salmo salar (Rudy and Potts 1969). The Chehalis Hatchery water supply is very soft (9.5-11.2 mg/L as $CaCO_3$), and it is known that waters low in Ca^{2+} may contribute to increased mortality in salmonid eggs and alevins, particularly at acid pH levels (pH 4.5) (Brown 1982). Sodium (0.8-0.9 mg/L) and potassium (0.07-0.13 mg/L) levels also are low in the Chehalis water supply, in comparison with waters that sustain native chinook populations (Harrison, Big Qualicum Rivers; Table 1, General Introduction). Low levels of calcium and sodium have been shown to lower survival in hatching and post-hatch Salmo trutta at lower pH values (Brown and Lynam 1981).

It is not known precisely what concentrations of hydrominerals are required for alevins to be able to extract sufficient amounts of the cations for continued development and survival, but we suspect the levels prevailing at Chehalis Hatchery may be near the lower limits permitting normal development and survival. In addition, the Chehalis Hatchery water supply is slightly acidic (pH 6.4-6.6). This would tend to accentuate the problem of cation absorption (McWilliams 1980; Peterson and Martin-Robichaud 1986) although threshold cation levels for normal physiological function are not yet known.

This study examines uptake rates for Na^+, K^+, Ca^{2+}, and Mg^{2+} by Harrison River chinook alevins reared at Chehalis Hatchery. Comparisons are made in alevins from the same source transferred to and raised at Chilliwack Hatchery, and with Capilano Hatchery (Big Qualicum River stock) alevins raised at Capilano Hatchery. The objective of this study was to determine whether or not there could be a relationship between the low hydromineral ion levels in the Chehalis Hatchery water supply and the problem of mortality among chinook alevins.

MATERIALS AND METHODS

The alevins in all locations were housed in 8-tray vertical incubators (Heath Techna Corp.) at a water flow through the trays of 12 L/min. Sampling of alevins at Chehalis Hatchery began on 20 January 1984 and was completed on 9 March 1984. During this period water temperatures varied between 6.2 and 4.2°C. Alevins were sampled at eight locations in the hatchery:

1K - alevins relatively unaffected by the problem throughout the sampling period; used as a control for
1J - alevins subject to Ca^{2+} injection ($CaCl_2$ drip system into the water supply) between 654 and 709 ATU in an attempt to alleviate mortality.
2K3 - "affected" comparison
2J, 2J3 - other "unaffected" locations
2K2, 2K4, 2K7 - other affected locations

At Chehalis Hatchery mortality occurred mainly between 750 and 870 ATU. No significant mortality occurred among the Chehalis alevins reared at the Chilliwack Hatchery. Capilano Hatchery alevins were sampled between 592 and 772 ATU.

A sample consisted of five alevins, frozen on aluminum foil. Alevins were then weighed individually, placed in pre-weighed glass vials, and oven-dried for 48 h at 60°C. Dry weights were determined by subtraction. The alevins were digested in conc. HNO_3 and the acid was evaporated at 60°C. The residue was redissolved in conc. HNO_3 and diluted appropriately for determination of Na^+, K^+, Ca^+, and Mg^{2+} content by AAS. A detailed description of the method is given elsewhere (Rombough and Garside 1983). U.S. National Bureau of Standards bovine liver standards were treated in an identical manner to evaluate the accuracy of the technique, and concentrations found were not significantly different from those certified (Table 1).

Table 1. Comparison of certified values for sodium, potassium, calcium and magnesium in U.S. National Bureau of Standards bovine liver samples with values determined in this study.

Cation	Units	Certified Values	Determined Values	Percent Recovery
Na	mg/L	2.43 ± 0.13	2.50 ± 0.06	102.9
K	mg/L	9.96 ± 0.07	10.13 ± 0.21	101.7
Ca	µg/L	120 ± 7	122 ± 16	101.9
Mg	µg/L	600 ± 15	601 ± 11	100.2

Hence, the values determined are considered as reliable estimates of actual cation concentrations per gram of dry material.

RESULTS

Observations and data relevant to alevin sampling are shown in Table 2. Regression relations between alevin age (ATU) and levels of Na^+, K^+, Ca^{2+}, and Mg^{2+} are shown, respectively, in Figure 1A, B, C, and D. The regression lines obtained exclude from computation those samples of Chehalis Hatchery alevins where significant mortality occurred.

SODIUM

The slopes of the regression lines relating sodium uptake and alevin age (ATU) are not significantly different for the three hatchery locations, Chehalis, Chilliwack, and Capilano (Fig. 1A). This suggests that uptake rates were unaffected by the ambient $[Na^+]$. However, alevin mortality occurred in Chehalis locations 2K2, 2K3, and 2K7 after 750 ATU, and it might be argued that ambient $[Na^+]$ could have limited uptake after 750 ATU. This assumption is not supported by samples from the control (1J), which continued to take up Na^+ at the control rate after 750 ATU. On the other hand, the elevation of the Capilano regression is significantly greater ($P<0.01$) than that for the Harrison fish at Chehalis or Chilliwack. These data could be interpreted in several ways: (1) the Capilano eggs could have begun (at the eyed stage) accumulating Na earlier or at a greater rate where ambient $[Na^+]$ was higher, and (2) Chehalis ambient $[Na^+]$ may not be limiting as the rate of accumulation of Na^+ by the Harrison alevins at Chilliwack, where ambient $[Na^+]$ is about eight times higher than at Chehalis (presumed not limiting), is the same as the rate of accumulation at Chehalis. It seems more likely that the Harrison eggs either had lower initial Na levels or began accumulating Na at a later stage. The former seems less likely, as

Table 2. Alevin sampling dates, water temperatures and sampling locations, Chehalis Hatchery.

Date sampled	Water temp. (°C)	Location	ATU	Comments	Location	ATU	Comments	Location	ATU	Comments
20/1/84	6.2	1J	654	Several dead	1K	652	Several dead	2J	609	No dead
23	6.2		672	Several dead		672	A few dead		628	No dead
26	6.2		691	Several dead		691	Very few dead		646	A few dead
29	6.1		709	Very few dead		709	Very few dead		664	A few dead
2/2/84	6.0		733	Very few dead		733	Very few dead		688	Very few dead
5	6.0		751	No new dead		751	No new dead		706	No new dead
8	5.5		769	No new dead		769	No new dead		724	No new dead
11	5.5		785	No new dead		785	No new dead		740	No new dead
15	5.3		805	-		805	-		760	No new dead
19	5.2		828	Very few dead		828	-		783	Very few dead
23/2/84	5.2	2K3	803	Unaffected	2K4	758	Lethargic	2K7	758	Many dead
25	4.7		813	Unaffected		768	Lethargic		768	Many dead
27	4.2		823	Unaffected		778	Many dead		778	Many dead
29	4.9		833	A few dead		788	Many dead		788	Many dead
2/3/84	5.0		843	Heavy loss		-	-		798	Complete mortality
2/3/84	4.4				2K2	843	Many dead			
4	4.5	2J3	853	Unaffected		853	Many dead			
7	5.3		868	Unaffected		868	Many dead			
9	4.9		872	Unaffected						

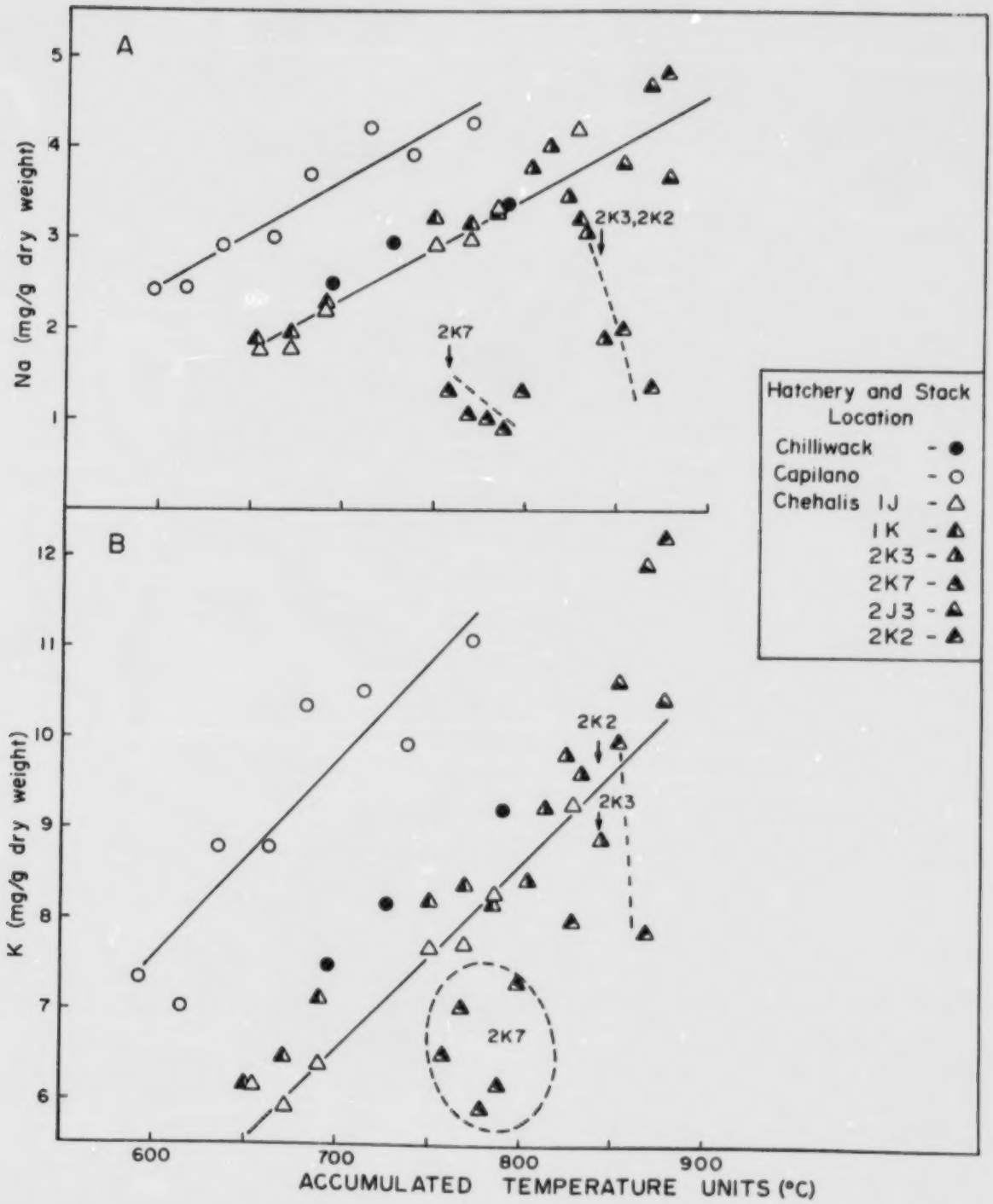

Fig. 1. Rates of uptake hydromineral ions by chinook alevins between about 600 and 875 ATU. A - sodium, B - potassium, C - calcium, D - magnesium (mg/g dry weight). Alevin groups compared are identified in the inset boxes. Regression lines identify the main relations or those differing significantly from each other. Groups showing significant mortality (2K2, 2K3, 2K7) are not included in the regression relations.

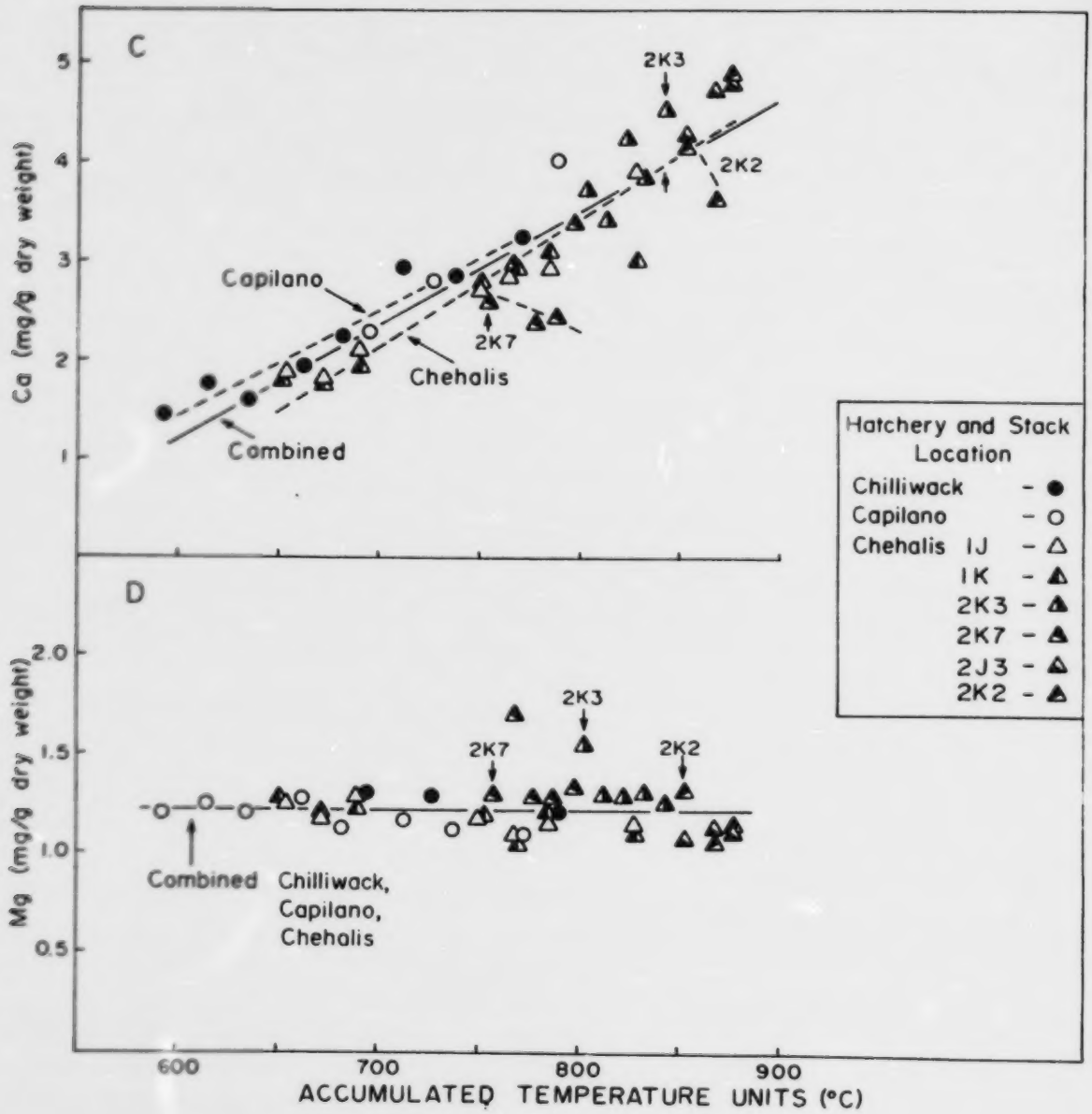
C
D
Ca (mg/g dry weight)
Mg (mg/g dry weight)
2K3
2K2
2K7
Capilano
Chehalis
Combined
Combined Chilliwack, Capilano, Chehalis
Hatchery and Stack Location
Chilliwack - ●
Capilano - ○
Chehalis 1J - △
1K - ▲
2K3 - ▲
2K7 - ▲
2J3 - △
2K2 - ▲
600
700
800
900
ACCUMULATED TEMPERATURE UNITS (°C)

Fig. 1. (cont'd)

the adults, prior to maturation and migration, would have had similar opportunities to accumulate Na in the marine environment. If Harrison eggs begin accumulating Na^+ at a later stage during incubation, this would seem to suggest potential genetic differences in the Harrison and Capilano stocks.

The regressions for sodium uptake are (mg/g dry weight)

(1) Capilano alevins

$$[Na^+] = 0.01139 \text{ (ATU)} - 4.3513 \qquad r = 0.95 \quad P<0.01$$

(2) Harrison alevins, excluding those groups showing significant mortality

$$[Na^+] = 0.01106 \text{ (ATU)} - 5.4138 \qquad r = 0.94 \quad P<0.01$$

In Figure 2A, sodium uptake is compared in group 1K (control, unaffected), in group 2K3 (affected) and in group 1J where calcium was injected into the incubator between 654 and 709 ATU. The comparison suggests that (1) something, possibly external $[Na^+]$, may have begun to limit Na^+ uptake at 750 ATU, (2) Ca^{2+} addition may have increased the rate of Na^+ uptake beyond 750 ATU, and that (3) Na^+ uptake had begun to decrease about 30 ATU (6 days) before heavy mortality began. However, it is not possible to determine (Figure 2A) whether the depressed sodium uptake in group 2K3 is a symptom of the influence of some pathological agent, or the result of an inherent inability to extract Na^+ from the medium at the concentrations encountered in the unmodified Chehalis Hatchery water supply.

POTASSIUM

The pattern of potassium uptake by the various alevin groups (Fig. 1B) is similar to that observed for sodium. Uptake rates of the Chehalis, Chilliwack, and Capilano alevins were not significantly different, but the elevation of the Capilano line is significantly greater ($P<0.01$) than that for the Harrison alevins at Chehalis and Chilliwack. Potassium concentration in the Chilliwack Hatchery water is at least eight times that at Chehalis (Table 1); hence, it is unlikely that $[K^+]$ would limit K^+ uptake at Chilliwack. An effect similar to that for sodium is seen in the reduction in rate of potassium uptake (Fig. 2B) in the control group (1K) after 770 ATU. Again the difference in elevation of the Capilano alevin regression line, for potassium uptake, suggests that potassium uptake may have been subject to the same conditions that influenced sodium uptake. That is, Harrison eggs may have had lower initial K^+ levels, or they may have begun accumulating K^+ at a later stage; the latter, as for Na^+, is assumed to be more probable. In group 2K3 (Fig. 2B) loss of potassium began about 20 ATU before heavy mortality occurred. Again, it is not possible to determine whether depressed K^+ uptake is a symptom of the action of a pathogen, or the result of an inability of the alevin to extract K^+ from the water at the concentrations present in the Chehalis water supply. It is possible that the low $[Ca^{2+}]$ in the Chehalis water supply in some way may have inhibited uptake of both sodium and potassium.

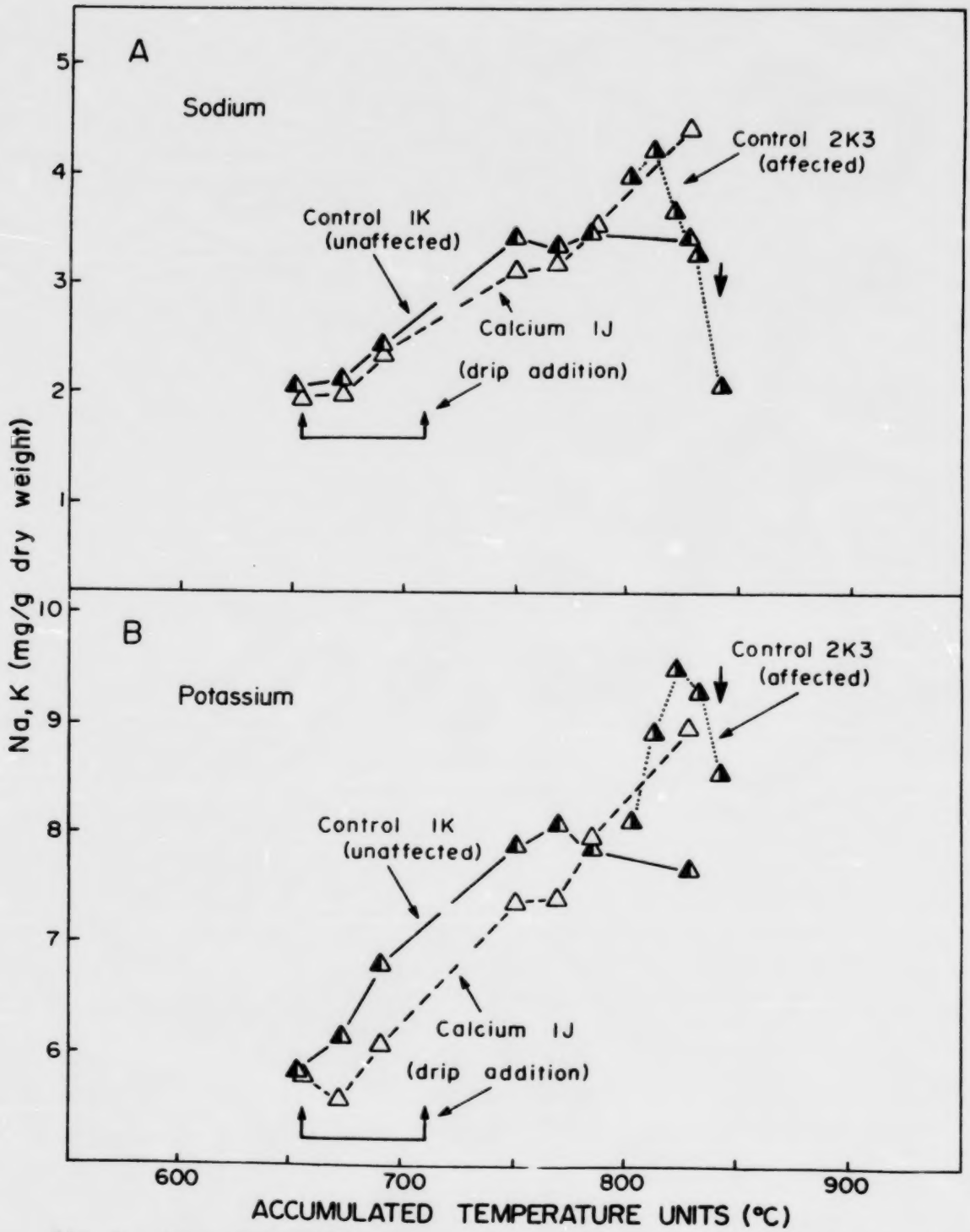

Fig. 2. Comparison of (A) sodium uptake, and (B) potassium uptake (mg/g dry weight) in three groups of chinook alevins at Chehalis Hatchery between 650 and 850 ATU. Group 1K - unaffected; group 2K3 - affected; and group 1J in which $CaCl_2$ was injected into the incubator between 650 and 709 ATU. Downward pointing arrows indicate when heavy mortality commenced (group 2K3). Note the change in uptake rates of Na^+ and K^+ occurring in group 1K near 750 ATU.

The regressions for potassium uptake are (mg/g dry weight)

(1) Capilano alevins

$[K^+] = 0.02194\ (ATU) - 5.6169$ $r = 0.91$ $P<0.01$

(2) Harrison alevins, excluding those groups showing significant mortality

$[K^+] = 0.02099\ (ATU) - 7.8865$ $r = 0.93$ $P<0.01$

CALCIUM

In contrast to sodium and potassium, there were no significant differences either in the slopes or elevations of the Capilano, Chehalis or Chilliwack regressions relating tissue $[Ca^{2+}]$ and alevin age (ATU) (Fig. 1C). Tissue $[Ca^{2+}]$ was depressed in some but not in all of the groups that suffered extensive mortality. The calcium results indicate that the differences in the Harrison and Capilano stock Na^+ and K^+ levels are not the result of some systematic analytical error. It also seems unlikely that mortality in the Harrison alevins is the result of calcium deficiency or imbalance. Hence, the earlier suggestion that calcium imbalance may have influenced uptake of Na^+ or K^+ seems unlikely. The argument seems to narrow to one in which the presence of a pathogen may be the primary problem, and that mortality may be a direct or indirect result of Na^+ and K^+ ion loss as part of a general breakdown in osmoregulation.

The regressions for calcium uptake are (mg/g dry weight)

(1) Capilano alevins

$[Ca^{2+}] = 0.01050\ (ATU) - 4.8607$ $r = 0.97$ $P<0.01$

(2) Harrison alevins, excluding groups showing significant mortality

$[Ca^{2+}] = 0.0129\ (ATU) - 6.8861$ $r = 0.95$ $P<0.01$

(3) Harrison-Capilano alevins (combined)

$[Ca^{2+}] = 0.01137\ (ATU) - 5.6326$ $r = 0.95$ $P<0.01$

MAGNESIUM

There is no significant trend in tissue Mg^{2+} levels during alevin development (Fig. 1D), nor are there any significant differences in magnesium levels in alevins from the three hatcheries.

The regression for magnesium is as follows (mg/g dry weight)

(1) Combined Capilano, Harrison and Chilliwack

$[Mg^{2+}] = 0.000046\ (ATU) + 1.1914$ $\quad r = 0.08 \quad P>0.05$

DISCUSSION

Water imbibition during the formation of the perivitelline fluid, following fertilization, essentially is largely completed in 1-2 h, and early egg volume changes are fully completed in about 20 h. Over this period the rate constant for water exchange in an egg declines exponentially (Potts and Eddy 1973) and the permeability of the vitelline membrane decreases as the hydrostatic pressure within the egg rises with imbibition (Hansson Mild and Lovtrup (1974). The $[Na^+]$ in the newly water hardened (~100 mg) S. salar egg is about 18-20 µM/g (41-46 µg Na/100 mg wet weight) (Rudy and Potts 1969; Eddy and Talbot 1985). In comparison a 250 mg chinook alevin at 650 ATU would contain about the same amount of Na^+ (about 1.75 mg/g dry weight, Fig. 1A), that is about 88 µg Na/250 mg wet weight (assuming embryo + yolk is 80% water). The perivitelline fluid has a substantial content of non-diffusible colloids (Eddy 1974); the excess of anionic charges these present tend to accumulate monovalent cations, such as Na^+, from the external medium (water) by a process similar to the Donnan effect. Hence the perivitelline fluid acts as an ion trap (Eddy and Talbot 1985), increasing $[Na^+]$ in the perivitelline fluid and reducing the concentration gradient for Na^+ across the vitelline membrane. Relative to Chehalis well water, $[Na^+]$ in the perivitelline fluid of the chinook egg should be about fivefold that of the prevailing $[Na^+]$ in the perfusing water (Rudy and Potts 1969). The reduced concentration gradient between the embryo and the perivitelline fluid should tend to reduce or inhibit Na^+ efflux from the embryo. From fertilization until the eyed embryo stage, evidence indicates that there is a slow net loss of Na^+ from the embryo and yolk. From the eyed stage through hatching and into the alevin stage there is a net gain of Na^+. For Salmo salar eyed eggs (Eddy and Talbot 1985) Na^+ influx rates were about 0.147 µg/g wet weight/hr; and efflux rates were 0.055 µg/g wet wt/hr, giving a net influx of 0.092 µg/g wet weight/hr. Net influx rates for dechorionated embryos were quite similar. Uptake of Na^+ in the Chehalis, Chilliwack and Capilano chinook alevins may be estimated (Fig. 1A) from the regression coefficients (0.01106-0.01139 mg/g dry weight/ATU). Assuming egg dry weight (embryo + yolk) is 20% of original wet weight, Na^+ uptake is estimated as 0.095 µg/g wet weight/hr -- equivalent to an estimate of net influx. The fact that the Atlantic salmon and chinook salmon estimates are almost identical is fortuitous. The Atlantic salmon eggs were from water of 0.1 mM Ca^{2+} (~4 mg/L Ca^{2+}), slightly greater than the $[Ca^{2+}]$ in the Chehalis water supply. Since the Chehalis and Chilliwack uptake rates of Na^+ were virtually identical, the $[Na^+]$ in the Chehalis water (0.8-0.9 mg/L) at approximately the same $[Ca^{2+}]$, would not appear to be limiting Na uptake.

The expanding "acid rain" problem in the northern hemisphere (McWilliams 1980) has brought increasing attention to bear on certain criteria having to do with water quality, including pH, Na^+, K^+, Ca^{2+}, and Mg^{2+}

concentrations, total hardness, and presence of aluminum and heavy metals. A number of these parameters are interrelated in natural waters, and limits or criteria for acceptable conditions are neither available as yet nor well understood. Rombough and Jensen (1985) noted for Salmo gairdneri eggs that water uptake and ability to resist deformation (hydrostatic pressure + capsule stiffness) were significantly affected at pH 5.5 and 6.0 respectively. In addition, the thresholds for these properties appear to be near pH 6.5. Conditions that influence water imbibition and apparent internal pressure are likely to be deleterious. Therefore, we suggest pH 6.5 as a preliminary criterion. There appears to be insufficient evidence as yet to allow an estimation of threshold requirements of Na^+ and Ca^{2+} in relation to pH. Brown and Lynam (1981) found that newly fertilized eggs of Salmo trutta at pH 4.5 survived better (10-30% mortality) at 10 mg/L Ca^{2+} than at 1 mg/L Ca^{2+} (92-100% mortality) over a 4-week exposure. Starting with eyed eggs at pH 4.5, survival was 80-100% at either Ca^{2+} concentration provided that $[Na^+]$ was 1 mg/L or greater. At the same pH, Brown (1982) further attempted to estimate Ca^{2+} requirements for S. trutta between 1 and 10 mg/L Ca^{2+}. We estimate from his probit plots that 50 and 95% survival should be attainable at $[Ca^{2+}]$ near 2-4 and 11 mg/L Ca, respectively, from the eyed stage onward. Using alevins of Salmo salar, Peterson and Martin-Robichaud (1986) show that a number of development and growth-related functions differed significantly between pH levels of 4.5 and 6.8, but not between 5.1 and 6.8. Nevertheless, there were trends involved, which would suggest thresholds could be located somewhere below pH 6.8. McWilliams (1980) shows that rates of Na^+ uptake in S. trutta vary at a given pH in relation to the origin of the stock and the environmental concentration of Na^+. For example, Na^+ uptake in a Norwegian hatchery stock was limited at pH 6.0-6.5 and ambient $[Na^+]$ of 5.75 mg/L. From Figures 1A, B, both Na^+ and K^+ uptake might appear concentration-limited after 750 ATU. Yet it is difficult to accept the assumption that background levels of Na^+ and K^+ should be found limiting to embryonic development at the same time -- unless, of course, the influence of one is dependent on the other, or both are influenced by another factor, such as $[Ca^{2+}]$.

One would expect the late alevins of Peterson and Martin-Robichaud (1986) to have accumulated about 9 µM K^+ (14.6 µg/mg dry weight) at an embryo dry weight of 24 mg. Similar Chehalis alevins (about 900 ATU) would have accumulated about 10-11 mg K/g dry weight. Although the K^+ content of the latter alevins is somewhat lower, it is of a similar magnitude as that for Atlantic salmon alevins of similar age.

Peterson and Martin-Robichaud (1986) found for alevins of Salmo salar that the $[Mg^{2+}]$ concentration at the end of the yolksac stage (28.8 µg) was about one-half the original quantity available in the yolk supply (60 µg). In this instance the $[Mg^{2+}]$ in the water supply (0.4 mg/L) is very similar to that in the Chehalis water supply (Table 1, General Introduction). Thus, the Mg^{2+} supply in the yolk appears to be in excess of larval requirements. Their terminal alevins contained about 1.3 µg Mg/mg dry weight; the chinook alevins in this study contained a similar amount, 1.23 µg/mg dry weight. We conclude that envinronmental $[Mg^{2+}]$ was not likely to be involved in the chinook alevin problem, and that the 0.3-0.4 mg/L of Mg^{2+} in the Chehalis Hatchery water supply is not a limiting factor.

In summary we make the following conservative estimates of hydromineral requirements during incubation of salmonid eggs, and suggest they be used as guidelines until further data become available.

pH: not less than 6.5; preferable range 6.5-7.0
Na^+: $\geq$1 mg/L
K^+: ~0.1 mg/L
Ca^{2+}: $\geq$10-11 mg/L
Mg^{2+}: not likely to be a problem
Total hardness (mg/L as $CaCO_3$) 2.497 x Ca^{2+} = minimum of 24.9

In the present instance there is substantial yet still circumstantial evidence that a pathogen is the primary instigator of the Chehalis Hatchery chinook alevin problem. From the water chemistry evidence reviewed we suspect that low levels of some hydromineral ions in the water supply may contribute to the problem, particularly Na^{2+}, K^+, and Ca^{2+}. It also is evident that some stocks of chinook salmon are more susceptible to the problem than others, and we suspect that between-stock differences in hydromineral ion accessibility may be involved in differential susceptibility. It follows that specific stocks of chinook salmon, in genetic equilibrium with waters of higher hydromineral content (e.g. Harrison River), may be more susceptible to the pathogen when incubated in waters of low hydromineral content (e.g. Chehalis Hatchery). Whether primary or secondary in nature, ionoregulatory failure does occur, apparently involving reduced influx or increased efflux of Na^+ and K^+ ions in particular. Carl and Healey (1984) reported on differences in enzyme frequency and body morphology in three juvenile life history types of chinook salmon -- one entering a high salinity estuarine environment immediately after emergence, the second rearing in fresh water for 2 months, and the third rearing for a year in fresh water. It would be of interest to examine the chronology of chloride cell development in these three genetic types and possible differences in their ionoregulatory capacities, particularly in fresh waters of low ionic content. Differences in ionregulatory capacity of the three genetic types in low ionic freshwater media could have an important influence on stocking policy involving transfer of stocks, in genetic equilibrium, between waters of different hydromineral content.

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INTRODUCTION

The Chehalis Hatchery has had continuing problems with mortality among recently hatched alevins of chinook salmon (Oncorhynchus tshawytscha). Earlier studies produced evidence that a pathogen is involved (Traxler and Rankin 1987), including progressive involvement of alevins in lower trays of an affected incubation stack, the development of typical symptoms among "unaffected" alevins when challenged with affected alevins, the presence of virus-like particles in tissue of affected alevins, and the fact that ultraviolet treatment of "contaminated" incubation water reduces the magnitude of the problem. A genetic link also has been identified (Withler 1987). In a comparison of Harrison River (H) x Capilano River (C) stock crosses, HxH alevins are most susceptible and CxC alevins are less likely to be affected. Furthermore, susceptibility is influenced markedly by the origin of the male parent, the H male conveying the greater potential for susceptibility.

During 1984-85 a search for solutions to the problem was enlarged to consider whether water quality could be involved. Accordingly, a series of trials was conducted (Dec. 1985-March 1986) at Chehalis Hatchery. A prior search of the literature indicated that low levels of Ca^{2+}, Na^{+}, and K^{+} in the hatchery water supply could be involved, and that at the usual pH of the water supply (6.4-6.6) there might also be an Al problem. Major parts of the head works and distribution system are constructed of bright, unpainted aluminum. At pH 6.4-6.6, ion species most likely to be present would be $Al(OH)_4^-$ and $Al(OH)_2^+$ (Burrows 1977). The ion species of major interest in acid rain research is Al^{3+}; however, this ion species is not likely to be found above pH 5.5. A test series was set up to examine the influence of Al in the water, and to determine the effects of adding Ca, Na, and K to the incubation water supply.

MATERIALS AND METHODS

Twelve 8-tray vertical incubator stacks (Heath Techna Corp.) were used in the trials. In 8 of the stacks a 2^3 factorial design was set up with three factors (Al, Ca^{2+}, and $Na^{+} + K^{+}$) at two levels (background

and increased). The two potential levels of the first factor (Al) were obtained by coating all bright aluminum surfaces in contact with the water with epoxy paint to inhibit the possibility of Al going into solution (Stacks 1-4). Responses in these stacks were compared with those in Stacks 5-8 where bright aluminum surfaces were left bare. In each stack the first, upper tray was left vacant. Crushed dolomite limestone [$CaMg(CO_3)_2$] was added to the next three trays in Stacks 3, 4, 7, and 8 (~13.6 kg/tray) to increase calcium concentration (second factor) in the incubation water. The third factor, a mixture of Na^+ and K^+ provided by agricultural grade NaCl and KCl dissolved in hatchery water, was delivered to the appropriate stacks (Stacks 2, 4, 6, 8) by dosing pumps. Essentially, the lower levels of Al, Ca^{2+} and Na^++K^+ were background levels in the hatchery water supply (coded -1 level), while those at the higher level (coded +1) represent additions. In the final four stacks (Stacks 9-12), Al was at the lower (-1) level, Ca^{2+} was at the higher (+1) level, and Na^++K^+ were added in a series of increasing concentration steps between (-1) (Stack 3, 12) and (+1) (Stack 4) in the hope of obtaining quantitative data on Na^++K^+ requirements. Concentrations of Na^++K^+ in the 100-L stock tanks, whose contents were delivered to the stacks by dosing pump, were adjusted to provide output for 1 week (+10%) before refilling was required.

Water samples for Al, Ca^{2+}, Na^+, and K^+ were analyzed by the EPS-DFO Water Quality Laboratory, West Vancouver, B.C. The design and results of water analysis over the experimental period are shown in Table 1. Two problems are seen on inspection of the table. First, analysis showed that there was virtually no difference in [Al] between the painted (-1) and unpainted (+1) sides of the design (Stacks 1-4 vs. 5-8). The analyses showed that no aluminum was entering the incubation water from the unpainted Al surfaces in the head works; hence [Al] in the water represented that in the normal, unmodified water supply. Second was a problem in the addition of calcium. The quantities of dolomite used were insufficient to provide more than a modest increase (0.13 mg/L Ca), equivalent to a rise of 0.6 mg/L as $CaCO_3$ on the (+1) side of the design. Hence, the Al contrast is absent and that for Ca^{2+} is minimal.

Treatment of test lots of chinook eggs began about 10 days prior to hatching. Eyed eggs from the general hatchery stock (shocked and picked at 300 ATU) were pooled, mixed and distributed among the stacks. Three trays of eggs (about 5500/tray) were inserted into each stack on 12 Dec. 85 at a state of development equivalent to 488 ATU and at a perfusion rate of 12 L/min. Hatching occurred between 23 Dec. (504 ATU) and 27 Dec. (535 ATU). Temperatures during the tests ranged from 9.1°C (12 Dec. 85) to 4.9°C (11 March 86).

This first series of trials was based on the assumption that the mortality problem, whatever its cause, could be triggered by osmotic stress resulting from the low levels of hydromineral ions occurring in the hatchery water supply. Hence, the first two trays of alevins in each stack were maintained throughout the test period (12 Dec. 85 to 1-11 March 86) (488-1018 ATU) to judge the effects of salt additions on subsequent survival. On 14 Feb. 86 (903 ATU) the third tray of alevins in each stack (Stacks 1-8) was challenged by inserting a tray of affected alevins into the third tray position, while moving the third tray to the next position below the affected

Table 1. Design, average concentrations of dissolved Al, Ca, Na, and K (mg/L), and total hardness (mg/L as $CaCO_3$) for the 12 treatments of chinook eggs at Chehalis Hatchery, Dec. 1985 - 14 Feb. 1986.

Stack	Tray	Treatment Al	Ca	Na	K	Hardness	Stack	Tray	Treatment Al	Ca	Na	K	Hardness
1	3	-1	-1	-1	-1		9	6	-1	1	-.5	-.5	
	4	-1	-1	-1	-1			7	-1	1	-.5	-.5	
	5	-1	-1	-1	-1			8	-1	1	-.5	-.5	
Av. (mg/L)		.006	3.37	0.81	0.10	10.0	Av. (mg/L)		.005	3.52	3.75	2.42	10.5
2	3	-1	-1	1	1		10	6	-1	1	0	0	
	4	-1	-1	1	1			7	-1	1	0	0	
	5	-1	-1	1	1			8	-1	1	0	0	
Av. (mg/L)		.006	3.43	10.0	4.65	10.1	Av. (mg/L)		.004	3.48	5.14	2.47	10.4
3	6	-1	1	-1	-1		11	6	-1	1	.5	.5	
	7	-1	1	-1	-1			7	-1	1	.5	.5	
	8	-1	1	-1	-1			8	-1	1	.5	.5	
Av. (mg/L)		.005	3.57	0.82	0.11	10.8	Av. (mg/L)		.007	3.50	8.40	3.98	10.4
4	6	-1	1	1	1		12	3	-1	1	-1	-1	
	7	-1	1	1	1			4	-1	1	-1	-1	
	8	-1	1	1	1			5	-1	1	-1	-1	
Av. (mg/L)		.005	3.55	11.47	5.42	10.7	Av. (mg/L)		.006	3.47	0.81	0.12	10.3
5	3	1	-1	-1	-1								
	4	1	-1	-1	-1								
	5	1	-1	-1	-1								
Av. (mg/L)		.006	3.39	0.85	0.12	10.0							
6	3	1	-1	1	1								
	4	1	-1	1	1								
	5	1	-1	1	1								
Av. (mg/L)		.006	3.39	9.82	4.62	10.1							
7	6	1	1	-1	-1								
	7	1	1	-1	-1								
	8	1	1	-1	-1								
Av. (mg/L)		.007	3.52	0.79	0.10	10.7							
8	6	1	1	1	1								
	7	1	1	1	1								
	8	1	1	1	1								
Av. (mg/L)		.005	3.49	10.42	5.05	10.5							

pH was measured on 23 occasions during the sampling period. Mean pH was 6.65, and the range was 6.2-6.95.

tray. Affected trays of alevins used in the challenge were used as they became available. They were not pooled; therefore if a disease organism were involved, particle density could have varied among the various challenge trays. The challenged trays (position 4) provided a further comparison with the unchallenged trays (positions 1, 2) in each stack. Hence both challenged and unchallenged trays were exposed to additions of salts for the entire period (12 Dec. 85 to 1-11 March 86), while the challenged trays were also exposed to affected alevins from 14 Feb. to 1-11 March 86. All trays in Stacks 9-12 remained unchallenged for the entire period.

Some mortality did occur in the unchallenged trays of alevins in three of the stacks. In Stacks 5 and 6, mortality began between 19-26 Jan. 86 (tray 3, Stack 5; trays 1, 2, Stack 6). Mortality in these trays was very high; therefore when challenge occurred on 14 Feb. 86, these trays were withdrawn and replaced with new trays of alevins from the general hatchery stock. The new trays were challenged in the same manner as in the other stacks. In Stack 7 there was an indication of incipient illness on 13 Feb. 86, the day prior to the insertion of the challenge trays. By 22 Feb. 86, these trays were highly affected, and mortality was heavy through the remaining part of the test period.

Samples of eggs and alevins from the 12 stacks were taken at intervals throughout the total period (Dec. 1985 - March 1986). Analyses were conducted to determine the concentrations of Ca^{2+}, Na^{+}, and K^{+}; the results are presented in the following paper (Alderdice and Hally 1987).

RESULTS

Mortality in the unchallenged (Stacks 1-12) and challenged (Stacks 1-8) trays of alevins is summarized in Table 2. As the $Na^{+}+K^{+}$ addition was treated as one parameter, analysis was begun on the 2^3 design with two levels (-1, +1) of the three factors (Al, Ca^{2+}, $Na^{+}+K^{+}$).

UNCHALLENGED ALEVINS

Because of the loss of replicates in Stacks 5 and 6, to simplify the analysis the number of replicates was standardized at two by averaging two replicates with similar responses at the low end of the mortality range (Stacks 9-12) (Table 2). An analysis of variance of arcsin-transformed data was performed, recognizing the limitations imposed by the zero to low differences between the -1 and +1 levels of Al and Ca^{2+} in the design. Of the main effects, only that associated with aluminum was significant. That is, there was significantly lower mortality in Stacks 1-4 (painted Al surfaces) compared with Stacks 5-8 (bare Al surfaces). However, as the amounts of aluminum in the water supplying these two sets of stacks were virtually identical, the higher mortality in Stacks 5-8 could not be attributed to the presence of aluminum. Therefore, mortality in these trials

Table 2. Mortality among unchallenged and challenged trays of chinook salmon alevins at Chehalis Hatchery. In Stacks 5 and 6, the third trays in each set suffered high mortality prior to challenge; a new tray of "unaffected" alevins from the general hatchery stock was used to replace each loss.

Stack	Replicate	Unchallenged (%)	Challenged (%)
1	1	0.7	48.8
	2	0.8	
2	1	0.6	13.9
	2	0.7	
3	1	0.6	20.2
	2	0.8	
4	1	0.4	8.6
	2	0.7	
5	1	0.7	74.6
	2	91.5	
6	1	86.8	42.5
	2	84.5	
7	1	73.7	39.1
	2	36.7	
8	1	0.6	1.8
	2	0.8	

must have had a different etiology. The only seemingly feasible explanation is to assume that the unchallenged alevins were responding to the presence of a pathogen from infected eggs at the beginning of the trials. That being so, then the absence of substantial mortality in Stacks 1-4, 8, 9-12 would suggest that these tray of alevins were free of, and remained free of pathogen, from the beginning of the trials. Hence, mortality in the unchallenged alevins would be a result of chance and random presence of pathogen. In addition, the conditions provided in Stack 5 (background, no additions), Stack 6 (higher $Na^+ + K^+$) or Stack 7 (higher Ca^{2+}) were insufficient to prevent illness in the unchallenged alevins in those trials. Presumably mortality also could have occurred in the remaining stacks if there had been a source of pathogen in those trays as well.

When Stacks 9-12 are considered, a series of six levels of $Na^+ + K^+$ are presented with Al (-1) and Ca^{2+} (+1). Prechallenge mortality in this series is shown in Table 3. This series supports the suggestion either (1) that higher $[Ca^{2+}]$ and $[Na^+ + K^+]$ may have had beneficial effects on survival of unchallenged alevins or (2) that the unchallenged trays of eggs in Stacks 9-12 were free of pathogen. The latter is the more likely assumption.

CHALLENGED ALEVINS

A similar analysis was employed with the trays of challenged alevins (Table 2). There were no treatment replicates in this series. Analysis indicated that none of the 2-factor or 3-factor interactions involving (Al, Ca^{2+}, $Na^+ + K^+$) was significant. Gathering these as an error term, no significant effects could be demonstrated regarding aluminum. However, Ca^{2+} and $Na^+ + K^+$ effects were significant, respectively, with ($F=12.12$, $0.01<P<0.05$) and ($F=14.24$, $0.01<P<0.05$). Response in the challenged alevins, in terms of percentage mortality, was lower at the higher levels of Ca^{2+} and $Na^+ + K^+$:

		Calcium	
		-1	+1
$Na^+ + K^+$	-1	62.6	29.8
	+1	28.5	5.2

These results indicate that:

(1) Al was not involved. Under the conditions prevailing at the Chehalis Hatchery, Al apparently does not dissolve from bright aluminum surfaces in contact with water. Even so, there was some evidence of pitting of Al surfaces. Therefore, there may be episodes when Al goes into solution, perhaps at times of lower pH. However, as the alevin problem did occur during these tests, that problem cannot be identified with episodes of low pH.

Table 3. Mortality among unchallenged alevins in a series of stepped increases in concentration of $Na^+ + K^+$ at the background (-1) level of Al, and the increased (+1) level of Ca^{2+} involving Stacks 9-12.

Stack	Al	Ca (mg/L)	Na + K	Unchallenged Mortality (%)
3*	0.005	3.57	0.82 + 0.11	0.6, 0.8†
12*	0.006	3.47	0.81 + 0.12	0.7
9	0.005	3.52	4.69 + 1.20	0.5
10	0.004	3.48	5.14 + 2.47	0.5
11	0.007	3.50	8.40 + 3.98	0.8
4	0.006	3.55	11.47 + 5.42	0.4, 0.7†

*Repeated trials

†Replicates

(2) Challenge mortality was reduced, but not eliminated in those trials with high Ca^{2+} and $Na^{+}+K^{+}$ concentrations. This was so, even though in the case of Ca^{2+} the concentration was only marginally higher.

DISCUSSION

MORTALITY IN UNCHALLENGED AND CHALLENGED ALEVINS

Analyses for Al (Table 1) showed average concentrations of 0.0055 mg/L on the painted (-1) side, compared with 0.006 mg/L on the unpainted (+1) side. By inspection of the analytical series, this difference is judged to be inconsequential; as well the 0.006 mg/L Al concentration appears to be well below one that would promote deleterious action (Burrows 1977; Muniz and Leivestad 1980; Eddy and Talbot 1983). We conclude that little if any aluminum was entering the water from the alumuinum surfaces in the head works, and the amount in the water would represent background levels in the water supply. An examination of Chehalis Hatchery water quality parameters from MacKinley (1984), and from current sampling, shows that heavy metals, particularly Cu and Zn, are well below those levels likely to cause problems. The possibility that some other factor was operating in parallel with the Al treatment (painted vs. unpainted surfaces) seems improbable. As the Al treatment was nonfunctional, one might expect to see similar responses among stacks in which Ca^{2+} and $Na^{+}+K^{+}$ concentration were identical (Stacks 1 and 5, 2 and 6, 3 and 7) (Tables 1, 2). This is not so. The only other explanation of the results that seems plausible is to assume the presence, at random, of trays of eggs with unrecognized centres of infection by an unknown pathogen.

If this assumption is accepted, which seems reasonable, then further questions may be asked of the results, both for the unchallenged and challenged trays of alevins. In the unchallenged trays, if levels of pathogen initially were higher in stacks 5, 6 and 7 than in stacks 1, 2 and 3, the differences in response between stacks 1 and 5, 2 and 6, and 3 and 7 could be explained. Then it follows that the Ca^{2+} and $Na^{+}+K^{+}$ levels were ineffective in modifying the response to the pathogen in stacks 5, 6 and 7, and that they would have been equally ineffective in stacks 1, 2 and 3 if pathogen levels also had been high initially in those stacks. In the challenged trays, the results tend to agree with this assumption: mortality is appreciably higher in stacks 5, 6 and 7 as well as in stacks 1, 2 and 3. The remaining stacks (4 and 8) are both at the (slightly) higher Ca^{2+} concentration and higher $Na^{+}+K^{+}$ concentration, and both show reduced levels of mortality. Hence if the potential for pathogen-induced mortality in stacks 4 and 8 is approximately the average for the other six stacks (39.9%) then it may also be assumed that the Ca^{2+} and $Na^{+}+K^{+}$ concentrations in these stocks was sufficient to modify and reduce the effect of the pathogen in these stacks. Conversely, as affected alevins were not pooled prior to use in

challenge trays, it is possible that quantitative differences may have occurred in the level of pathogen presented. Thus, some treatments showing reduced mortality may have been responding to lower pathogen particle density rather than hydromineral addition.

The alevins in stacks 9-12 were not challenged; mortality remained very low throughout the test period and all trays of alevins from these stacks were considered "healthy" and were ponded 1 March 1986. The very low mortality rates in these stacks could have resulted from the treatments provided, or from levels of pathogen that were too low in these stacks to cause mortality. The latter seems more likely, for the following reason. Although stack 3 mortality in unchallenged trays was low (0.6, 0.8%; Table 3), unchallenged mortality in stack 7 was substantially higher (73.7, 36.7%, Table 2), yet treatments in these stacks, discounting any Al effect, were identical.

RELATED ARGUMENTS

The manner in which the crushed dolomite was used resulted in much lower increases in dissolved Ca^{2+} than anticipated, averaging 3.40 mg/L in the lower level and 3.53 mg/L at the higher level. The potential for real change in survival in relation to $[Ca^{2+}]$ may not have been realized under these circumstances; this is evident if the preliminary estimate of Ca^{2+} requirement applies as recommended by Alderdice and Rombough (1987) (10-11 mg/L Ca). The estimate of 10-11 mg/L Ca^{2+} is conservative; it is based on findings of Brown and Lynam (1981) and Brown (1982) for Salmo trutta at pH 4.5.

It has been shown (Rosseland et al. 1986) that the addition of Ca^{2+} to natural waters containing toxic amounts of Al can detoxify those waters with respect to both Al and pH. Yet in the present situation, neither Al or pH can be considered deleterious. It is obvious from other investigations that salmonids, at a pH of 4.5, tend to suffer ion losses and succumb from ionoregulatory problems (McWilliams 1980; Muniz and Leivestad 1980; Brown and Lynam 1981; Brown 1982; Eddy and Talbot 1985). Peterson and Martin-Robichaud (1986) compared hydromineral ion levels in Atlantic salmon alevins at three pH levels: 4.5, 5.1, and 6.8. Differences in uptake and loss rates suggest that thresholds for pH effect are somewhere between 5.1 and 6.8. We suspect, therefore, that the influence of pH per se in the current trials at pH 6.4-6.8 would be marginal at worst.

In the present situation, we favour the hypothesis that Ca^{2+} and Na^{+} levels in the Chehalis water supply are sufficiently low to place the alevins under ionoregulatory stress. Yet the bulk of the available data remains inferential. Rudy and Potts (1969) found that Atlantic salmon eggs used stored Na^{+} to the eyed stage; thereafter they accumulate environmental Na, and therefore they might suffer Na^{+} deprivation in low Na^{+} environments. Peterson and Martin-Robichaud (1986) determined hydromineral budgets for Atlantic salmon alevins. They found that the amounts of Ca^{2+}, Na^{+}, and K^{+} that must be obtained from the ambient medium for normal requirements were: Ca^{2+}, 85%; Na^{+}, 70%; and K^{+}, 40%. Eddy and Talbot (1985) found that the newly fertilized Atlantic salmon egg (2.41 µM Na/egg) may show a net sodium loss during development to the eyed stage (1.84 µM

Na/egg). Thereafter the embryo likely withdraws Na^+ from the perivitelline fluid, which itself presumably sequesters further Na^+ from the external medium by a Donnan effect. In eggs of Salmo trutta at pH 4.5, Brown and Lynam (1981) found mortality rates from fertilization to hatching in average Ca^{2+} levels of 0, 0.92, and 9.51 mg/L were, respectively, 100% in 5-8 d, not less than 90% in 19 d, and 10-33% in 26 d. They also found that average environmental Na^+ levels of 0, 0.85, and 1.93 mg/L had little if any influence on survival during incubation. Their control (18 mg/L Ca, 10 mg/L Na) mortality was 12% in 26 d. In a second experiment, starting with eyed eggs under similar conditions, 30% of the alevins died during hatching in the low Ca^{2+} level, and medium survival time among the survivors was 10 d. At the intermediate Ca^{2+} level, 80% hatched and median survival time was 30 d. In the high Ca^{2+} trials, 95% hatched and median survival time was again 30 d. In the low Na^+ trials, median survival time was 14 d; in the medium and high Na^+ trials it was 30 d. Thus, in the first set of trials from fertilization to the eyed stage, survival comparable to that of the controls was obtained with 9.51 mg/L Ca^{2+} and $[Na^+]$ was without effect. However, in the second set from the eyed stage onward, greatest survival to hatching occurred in 9.51 mg/L Ca^{2+}, and greatest post hatching survival occurred where ambient [Na] was 0.85-1.93 mg/L. Brown (1982) held Salmo trutta eggs from fertilization through hatching at pH 4.5 in waters intermediate in $[Ca^{2+}]$ to those of Brown and Lynam (1981). Ca^{2+} concentrations providing 50% survival were a) 2-2 1/2 mg/L to the eyed stage, b) 3 1/2 mg/L to hatching and c) 3 3/4 mg/L to swim-up at 90 d. However, examination of the probit lines involved indicated that 95% survival to the eyed, hatched and swim-up stages would require 10-11 mg/L Ca^{2+} in the ambient medium. Swinehart and Cheney (1984) exposed eggs of chinook salmon to waters of pH 5.0; the eggs showed a small net efflux of Ca^{2+} and Mg^{2+} exceeding that occurring in distilled water. Skogheim and Rosseland (1984), using eggs of Salmo salar, found mortality rates were very low in their controls at pH 6.4-6.8, Ca^{2+} levels of 3-4.5 mg/L and Al levels <0.010 mg/L, conditions not unlike those at Chehalis Hatchery.

Of considerable interest is a study of hydromineral requirements of juvenile Salmo gairdneri (Bradley and Rourke 1985). In waters at pH 6.6-7.1 with 1.2-1.6 mg/L Na^+, 0.4-0.7 mg/L K^+ and 3-4 mg/L Ca^{2+}, substantial mortality occurred, peaking 40-50 d after initiation of the tests. Extensive gill swelling began about day 27 and feeding activity dropped markedly on day 29. Mortality rate increased dramatically thereafter. Mineral additions began on day 43; after day 47 the mortality rate decreased rapidly to day 60. The hydrominerals added on day 43 provided final concentrations of 20 mg/L Na^+, 5 mg/L K^+ and 30 mg/L Cl^-. A significant increase in plasma ammonia occurred between days 27-40, which coincided with changes in gill structure and mortality. Gill swelling was evident on day 27, particularly in lamellae in the upper one-third of the gill filaments; edema and lamellar fusion where prevalent. None of these observations occurred in the controls. The authors hypothesized that low environmental $[Na^+]$ reduced NH_4^+ excretion by a Na^+/NH_4^+ exchange mechanism.

The reported studies indicate that eggs and alevins may respond to complex environmental alterations in which some or all of the factors considered (pH, Ca^{2+}, Na^+, Al, and possibly K^+) may be involved.

Evidence is available at pH 4.5 on a number of aspects of egg development, but little is available at pH levels around neutrality. The data of Rombough and Jensen (1985) on newly fertilized eggs of Salmo gairdneri suggest that a threshold for normal uptake of water during imbibition, and development of internal pressure, exists near a pH of 6.5. From fertilization to the eyed stage, the evidence suggests that Na^+ and K^+ stores are sufficient to satisfy developmental needs. From the eyed stage on, Na^+ and K^+ appear to be taken up from the external medium. Therefore, beyond the eyed stage we suspect that pH should be >6.5, $Na^+ \geq 1$ mg/L and $Ca^{2+} \geq 10$ mg/L in the ambient water supply.

Some consideration may be given to the manner in which these water chemistry variables can influence physiological processes. Marshal (1985) shows that the function of the opercular integument in fishes appears to involve resistance to the transport of ions across its surface. Normal function involves the blocking of paracellular pathways to ion transport by tight junctions at the apical margins of the epithelial cells. Increased ion loss across the integument in acid pH may result from the removal of Ca^{2+} from the tight junctions, which is required for their maintenance and integrity. With reference to Na^+, Aronson (1985) reviews the properties of the Na^+-H^+ (plasma membrane electrically neutral) ion exchanger, which can mediate Na^+-H^+, Na^+-Na^+ or Na^+-NH_4^+ exchanges. For example, reduced external pH tends to inhibit exchange of external Na^+ or internal H^+; external NH_4^+ also tends to stimulate Na^+ efflux and inhibit Na^+ influx. The system is dependent on internal $[H^+]$ and may play an important role in intracellular pH regulation (Heisler 1980, 1982).

Laurent and Dunel (1980) examined tissue development in the gills of various fishes in saltwater and deionized water. In early development of the gills there are two initial tissues, the primary and secondary epithelia. The former covers the primary lamellae and the interstitial region; the latter covers the free parts of the secondary lamellae. At hatching, in Salmo gairdneri, the embryonic primary lamellae are enclosed in pavement cells (epithelium) with interspersed and fully developed chloride cells. Chloride cells are not found in the epithelium of the secondary lamellae. In fresh water the chloride cells are single; there are no accessory chloride cells. In the rainbow trout and the eel (Anguilla anguilla) transferred to deionized water, there is an increase in chloride cells in the secondary lamellae. In the trout transferred to deionized water there is an initial intensive ion loss, and a proliferation of chloride cells; apparently, loss of calcium stimulates ion flux. It appears that proliferation of chloride cells in the secondary lamellae may provide a compensatory mechanism for increasing net ion uptake, particularly in waters of very low ionic strength. By analogy, these circumstances could apply to the Chehalis alevins.

Carl and Healey (1984), working with wild chinook salmon, showed there are three subpopulations of juveniles in the river studied (Nanaimo River) with respect to allelic frequencies that are associated with different juvenile life history types. These subpopulations vary in their timing of entry into estuarine seawater, and we wonder if they may also vary in their

ability to accumulate hydromineral ions in fresh water. Different spawning populations might show different proportions of the three subpopulation types in relation to the subpopulation strategy proven most successful for a chinook stock untilizing a given spawning river. One might assume that a given allelic frequency (life history type) is associated with early development of the Na^+/H^+ or Na^+/NH_4^+ ion exchange system or of extralamellar or lamellar chloride cells. A life history type without this early ionoregulatory capability could be less capable of adapting to early migration to sea water or to residence in low ionic strength fresh water. Again, by analogy, there could be a link between subpopulation type (Harrison River fish genetically adapted to higher ionic strength fresh water) and susceptibility to ionoregulatory problems in low ionic strength (Chehalis) fresh water.

The influence of acid or marginally low pH and low hydromineral ion content of the incubation water on embryonic and postembryonic (alevin) development may be related to the concurrent studies of Traxler and Rankin (1987) and Withler (1987). There seems little doubt that a pathogen is involved with the mortality associated with the challenge trials. Susceptibility to the as yet unidentified pathogen varies between stocks. The Harrison River male chinook contributes substantially to increased susceptibility to the Chehalis alevin mortality problem. If there were a direct relationship between susceptibility to the Chehalis problem and the ionic strength of the water with which a stock might be considered to be in equilibrium, then one might expect the stock from the highest ionic strength water (Big Qualicum or Capilano wells) (Table 1, General Introduction) to be at greatest disequilibrium with the low ionic strength water of Chehalis. This is not so: the male from the intermediate ionic strength water (Harrison River) contributes the most to increased susceptibility in terms of Chehalis hatchery alevin mortality (Withler 1987). Obviously, the total problem is not yet understood.

Whatever the components of the problem may be we are led to present the following hypothesis. The Chehalis hatchery chinook alevin problem appears to be triggered by the presence of an unknown pathogen, possibly endemic. Susceptibility to the problem varies genetically between stocks from different watersheds. It appears that genetic susceptibility may be related in some way to ionoregulatory stress resulting from low physiological levels of hydromineral ions in the incubation water supply, particularly Ca^{2+} and Na^+. Whether it is a primary or secondary symptom, affected alevins show rapid osmotic failure (efflux of Na^+, K^+) prior to death. We wonder whether the problem is associated genetically with the function or timing of initial function of the Na^+/H^+ or Na^+/NH_4^+ ion exchange system, as affected alevins at Chehalis hatchery show multiple symptoms of ammonia intoxication or retention. We wonder also if the presence or timing of development of chloride cells in the secondary lamellae, demonstrated by Laurent and Duvel (1980), may vary between stocks. If this applies to chinook salmon, then alevins with the capacity for earlier or more extensive chloride cell development should be able to adjust to and occupy waters of low ionic activity with greater capability for ionoregulation.

Finally, one is led to speculate that transfer of chinook stocks between watersheds should be done with care and reservation, particularly if different juvenile life history types (Carl and Healey 1984) are found to be associated with variations in early iono-regulatory ability in fresh waters of low ionic activity. In this regard we recommend provisionally, until further data are available, that chinook stocks should not be transferred to or incubated in water systems where pH is below 6.5, and where Ca^{2+} and Na^{+} concentration are below 10-11 mg/L and 1 mg/L, respectively. This level of Ca^{2+} may be too conservative, as it is based on requirements at pH 4.5. We speculate that a total water hardness of 20 mg/L as $CaCO_3$ is a reasonable provisional limit for incubation of chinook. This translates to a minimum value for Ca^{2+} of 8.01 mg/L. As for K^{+}, there is little quantitative data available; at this time we would recommend that $[K^{+}]$ be maintained at a minimum of < 0.1 mg/L.

ADDENDUM

A further examination of the influence of hydromineral ions on the Chehalis Hatchery chinook alevin problem was undertaken during December 1986-March 1987. In view of the problem of low Ca^{2+} concentration encountered in the previous year, Ca^{2+} concentrations were established using $CaCl_2$ instead of crushed dolomite. A resumé of the 1986-87 trials and results follows.

Twelve stacks of vertical incubation trays were loaded with pre-hatch chinook eggs, six trays per stack, on 18 December 1986. Eggs from three different pooled lots (3200-3400 per tray) were loaded in tray positions 2 and 6, 3 and 7, and 4 and 8, respectively in each stack. On 5 January 1987, trays of "affected" alevins were inserted in tray position 5 in each stack. The upper three trays (2, 3, 4) continued as unchallenged controls; the lower three trays (6, 7, 8), below the trays of affected alevins, served as challenged alevins. Surviving alevins were ponded 12 February 1987.

The twelve stacks were set up to examine the influence of raised levels of $[Ca^{2+}]$ and $[Na^{+}]$ on survival of the chinook alevins exposed to the Chehalis chinook alevin problem, now strongly suspected as being pathogenic in origin. Most trials were conducted at a raised level of K^{+}. Previous trials suggested that K^{+} has no influence on survival of chinook alevins exposed to the Chehalis problem. The 1986-87 trials provided an opportunity to check this assumption. The 12 trials consisted of 9 orthogonal trials of a 3^2 factorial design, with 3 additional trials to check the influence of $[K^{+}]$ at reduced levels. The design and nominal levels of Ca^{2+}, Na^{+}, and K^{+} used are shown in Table 4. Average concentrations of the ions actually delivered (18 December 1986-12 February 1987), and survival levels for each trial, are illustrated in Table 5.

The possible presence of pathogen in the control trays presents a complication. Estimates of control mortality can be obtained from the challenged trays (6, 7, 8) before challenge (5 Jan. 87) and from the

Table 4. Experimental design for 1986-87 trials involving additions of Ca^{2+}, Na^{+} and K^{+} to the Chehalis Hatchery water supply, and their influence on survival of chinook alevins exposed to the Chehalis "chinook alevin problem". Coded levels are shown for the three ions added.

Stack (Trial)	Coded levels Ca^{2+}	Na^{+}	K^{+}
1	-1	-1	1
2	-1	0	1
3	-1	1	1
4	0	-1	1
5	0	0	1
6	0	1	1
7	1	-1	1
8	1	0	1
9	1	1	1
10	0	0	-1
11	0	0	0
12	-1	-1	-1

Coded level	Ca^{2+}	Na^{+}	K^{+}
-1*	3.4	0.85	0.07
0	5.7	2.1	0.29
1	8.0	5.0	0.50

*Approximate background levels - Chehalis Hatchery water supply.

Table 5. Design levels and average concentrations of Ca^{2+}, Na^{+} and K^{+} occurring in the 12 incubation stacks involving hydromineral additions to the water supply, Chehalis Hatchery, 1986-87. All unchallenged trays showed minimum mortality (mean, 0.11%; range, 0.0-0.59%), but high mortality, indicative of the presence of pathogen, occurred in Stack 3 in the unchallenged trays (2, 3, 4) during the challenge period. These could have influenced mortality in the challenged trays (6, 7, 8). (See text)(R=replicate).

Stack 1 (trial)	Design level and concentrations Ca^{2+} (mg/L)	Na^{+} (mg/L)	K^{+} (mg/L)	Mortality (%) Unchallenged R_1	R_2	R_3	Challenged R_1	R_2	R_3	Mean
1	(-1) 3.4	(-1) 1.5	(1) 0.87	0.12	0.03	0.06	96.7	95.2	97.5	96.4
2	(-1) 3.4	(0) 2.1	(1) 0.39	0.0	0.0	0.12	93.2	91.1	95.1	93.4
3	(-1) 3.4	(1) 4.2	(1) 0.45	0.0	89.0	98.1	98.1	95.5	98.2	97.2
4	(0) 4.6	(-1) 1.2	(1) 0.33	0.12	0.09	0.0	88.4	94.1	92.4	91.7
5	(0) 6.0	(0) 2.0	(1) 0.40	0.0	0.0	0.03	93.9	90.1	93.1	92.6
6	(0) 5.4	(1) 5.3	(1) 0.62	0.50	0.15	0.09	71.1	89.8	90.8	91.1
7	(1) 7.2	(-1) 1.2	(1) 0.42	0.06	0.03	0.0	90.4	91.5	92.1	91.3
8	(1) 7.0	(0) 2.7	(1) 0.67	0.0	0.03	0.0	84.6	88.3	90.7	87.9
9	(1) 7.9	(1) 7.1	(1) 0.68	0.0	0.0	0.09	81.0	79.3	81.3	80.5
10	(0) 4.6	(0) 3.3	(-1) 0.07	0.0	0.0	0.0	91.6	91.7	96.6	93.3
11	(0) 5.3	(0) 1.9	(0) 0.29	0.0	0.0	0.0	89.4	90.3	94.0	91.3
12	(-1) 5.3	(-1) 1.1	(-1) 0.07	0.0	0.0	0.0	98.8	95.0	98.6	97.5

unchallenged trays (2, 3, 4) both before and after the challenge date. With the exception of Stack 3, control mortality was low (mean, 0.11%; range, 0.0-0.59%) from assumed non-pathogenic sources. However, several days after the challenge date, mortality in the unchallenged controls of Stack 3 began to increase rapidly from its origin in Rep 2 (Table 5). By the date of ponding, mortality in Reps 2 and 3, Stack 3, was nearly complete. Since these presumably infected trays were located above the test trays (6, 7, 8), they, together with challenge tray 5, would act as a source of infection to the test trays below them. In a sense, the timing was fortuitous: the test trays in Stack 3 were exposed to infection near but several (2-3) days before the challenge date. The longer challenge (2-3 days) and possible increase in pathogen particle density from the infected trays, may have resulted in some excess mortality in the Stack 3 test trays. We conclude that mortality in the challenged trays of Stack 3 may be overestimated by several percent. However, the remaining stacks appear to be free of this problem.

The results (Table 5) next were subjected to analysis of variance (Table 6). It is clear from the analysis that survival increases at higher concentrations of both Ca^{2+} and Na^{+}. The highly significant interaction between Ca^{2+} and Na^{+} shows the need for joint consideration of both ions: the level of response to one ion will depend on the concentration of the second ion applied simultaneously. A comparison of trials 10, 11, and 5 (Table 4; coded levels of Ca^{2+}=0, Na^{+}=0, K^{+}=-1, 0, 1) shows that $[K^{+}]$ likely has no influence on the response. The same results is indicated in trials 12 and 1 (coded levels of Ca^{2+}=-1, Na^{+}=-1, K^{+}=-1,1), where trial 12 is a control at background levels of the three ions in the hatchery water supply. Average survival in trials 12 and 1 is about 3%. Average survival in trials 10, 11, and 5 is higher (7.6%) likely a result of the fact that both Ca^{2+} and Na^{+} are at intermediate levels of concentration.

The ANOVA results suggested strongly that a response surface analysis be undertaken to examine the relations between $[Ca^{2+}]$, $[Na^{+}]$ and response (% survival) (Schnute and McKinnell 1984). The result (Fig. 1) shows a rising ridge in the Ca^{2+} -Na^{+} test space with its main axis rotated downward to the left, the major axis of the ridge running from lower left to upper right. An appreciation of the interaction between the two ions therefore can be demonstrated by showing a series of Ca^{2+} -Na^{+} concentration points on the major axis, and the corresponding survival values estimated to occur at those ion combinations. This is done in Table 7 for a range of $[Ca^{2+}]$ and $[Na^{+}]$ close to and within the boundaries of the test space in Figure 1. The data in Table 7 indicate that there is no single threshold concentration of Ca^{2+} or Na^{+} in relation to survival under the conditions imposed by the Chehalis chinook alevin problem. The protection afforded by the presence of higher concentrations of Ca^{2+} and Na^{+} is substantial, resulting in an increase in survival from background levels of Ca^{2+} and Na^{+} of about 3% to about 20% (trial 9). Presumably further increases in survival could be achieved by increasing the concentrations of the two ions further.

Although increased concentrations of Ca^{2+} and Na^{+} resulted in significant increases in survival of chinook alevins prior to ponding, there remains the question of whether the protection afforded is of practical value. The value of injecting salts into the whole hatchery incubation water

Table 6. Analysis of variance of the challenge data of Table 5 for chinook salmon alevins exposed to "affected" alevins, Chehalis Hatchery, 1986-87.

Source of variance	Df	S of S	MS	F
Calcium	2	371.42	185.71	55.2***
Sodium	2	57.96	28.98	6.63**
Ca x Na Interaction	4	149.42	37.36	11.12***
Error	18	60.49	3.36	
Total	26	639.30		

**0.01>P>0.001; ***0.001>P

Table 7. 1986-87 Chehalis Hatchery chinook alevin trials. Computed concentrations of Ca^{2+} and Na^{+} that would maximize survival of chinook alevins challenged by exposure to alevins affected by the "chinook alevin" problem. Relations computed by response surface analysis of the data in Table 5.

Ca^{2+} (mg/L)	Na^{+} (mg/L)	Est. survival to Swimup (%)
3.0	1.0	3.5
4.0	2.9	5.8
5.0	3.9	8.8
6.0	4.8	12.5
7.0	5.5	17.0
8.0	6.2	22.2

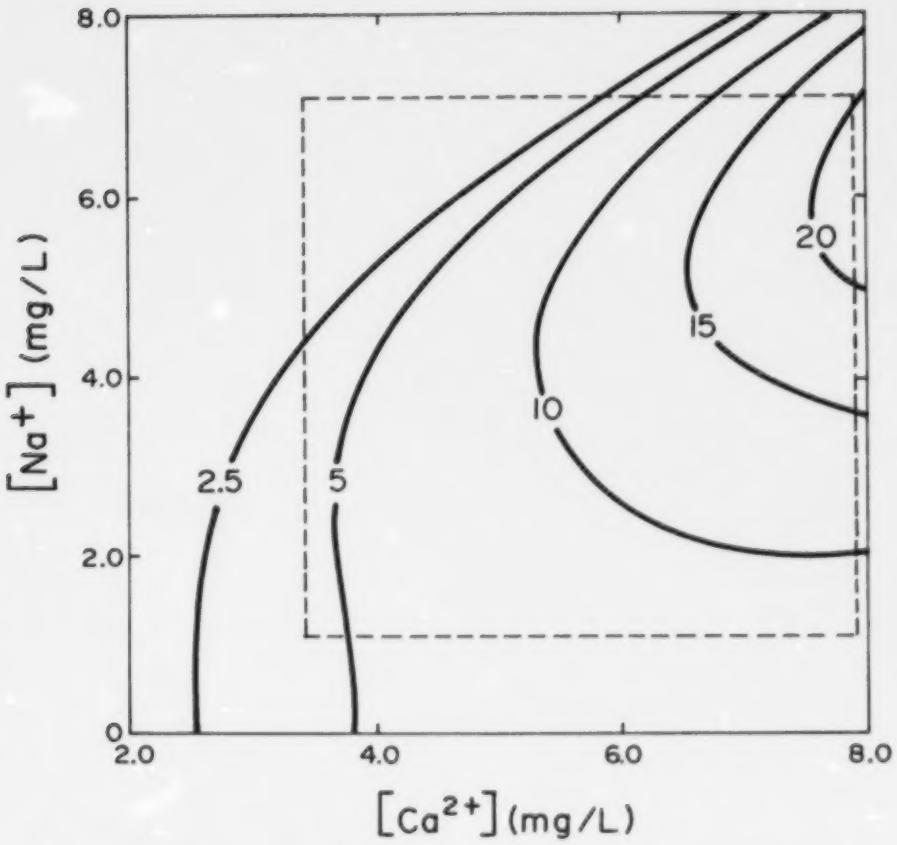

Fig. 1. Response surface showing computed relations between $[Ca^{2+}]$ and $[Na^{+}]$ in terms of response (survival %) of chinook salmon alevins exposed to the Chehalis Hatchery "chinook alevin" problem. The rectangle in the figure refers to the area encompassed by the 3^2 design (Table 4). The isopleths show expected percentage survival.

supply, at concentrations equal to or exceeding the maximum amounts used in these trials, would seem questionable. This is particularly so when the survival rate afforded to ponding (20%) would be well below that anticipated under normal conditions (~99%). Therefore, the amount of protection afforded seems insufficient. In addition, there remains a question of whether the naive, unaffected survivors remain susceptible after being ponded into the normal (low ion) hatchery water supply. Although survival among the control (unchallenged) groups (Table 5) remained near 99%, there were problems in several stacks. Control alevins in stack 6 showed initial symptoms of illness a few days before ponding on February 12. After ponding, mortality increased and survival in that group ultimately was about 16.7% (Feb. 28). The remaining unaffected tray of alevins from stack 3 (Table 5, trial 3, Rep 1) was ponded downstream from the survivors of stack 6: 9-10 days later these fish showed initial signs of illness, and mortality in the group increased thereafter. In addition, the problem inadvertently was introduced into the unchallenged fish from stack 5, some time after ponding, resulting in heavy mortality. Hence naive fish that avoid the Chehalis problem by chance while in the incubators, remain susceptible after ponding.

Generally, it is recognized that incubation and culture of salmonid eggs and alevins in soft waters is likely to encounter more life-threatening physiological or pathological problems than in hard waters. Yet, the function and requirements of hydromineral ions in either of these circumstances is poorly understood. We presume the stresses imposed on incubating eggs and alevins resulting from abnormal physiological or pathological conditions can have differing etiologies. We have no further cause to question our estimates of physiological threshold concentrations of hydromineral ions given earlier. However, these thresholds are likely to be basically different from requirements during pathological events. In this regard, we presume the low levels of Na^+ and K^+ noted by Alderdice and Rombough (1987) and Alderdice and Hally (1987) in "affected" fish represent abnormal tissue effluxes resulting from interference with normal ionoregulatory functions influenced by the presence of a pathogen. It appears that further investigation of the Chehalis Hatchery Harrison chinook alevin problem lies in a continuing search for the unknown pathogen, the most likely source of the problem.

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INTRODUCTION

From the previous papers in this issue (Traxler and Rankin 1987, Withler 1987, Alderdice and Rombough 1987, Alderdice et al. 1987) it appears that there are at least three components contributing to the problem of alevin mortality in chinook salmon (Oncorhynchus tshawytscha) at Chehalis Hatchery. These comprise a) the presence of a pathogen, as yet unidentified, b) genetic differences in susceptibility to the pathogen associated with origin of the stock and sex of the adult spawner, and c) influence of water quality parameters, particularly Na^+ and Ca^{2+} and possibly K^+, on the development and severity of the problem.

The original design in this study (Alderdice et al. 1987) was set up to evaluate the influence of Al, Ca^{2+} and $Na^+ + K^+$ on the alevin problem. Aluminum was proven not to be involved. Although difficulties also were experienced in establishing the intended higher Ca^{2+} level in the design, the authors were able to show that increased levels of Ca^{2+} and $Na^+ + K^+$ reduced mortality in trays of "affected" alevins. Difficulties were encountered in quantifying the results because of the unknown nature of the pathogen. That is, variations were assumed in the presence, absence or differences in density of the infective particles in the trials, both before and after challenge.

While the main trials involving water quality (Alderdice et al. 1987) were underway (1985-86 results), a series of samples of alevins was taken from the test trays at intervals (in accumulated temperature units, ATU) before (755.3-898.5 ATU) and after (959.0-963.6 ATU) challenge at 903 ATU (Table 1). From these samples the amounts of Na^+, K^+, and Ca^{2+} in the alevin tissues were determined for comparison with the concentrations of the three cations in the various water supplies to which the alevins were exposed in the design trials. This paper presents the results of those analyses.

MATERIALS AND METHODS

The modified design, minus the original Al contrast in Alderdice et al. (1987), is shown in Table 1. Twelve stacks of salmonid egg incubators (Heath Techna Corp.) were used, with a water flow of 12 L/min through each

Table 1. Summary of experimental design from Alderdice et al. (1987), collapsed into pairs of trials (Stacks 1-8) based on the lack of an Al effect in the original trials. Stacks (3,7,12), (9), (10), (11), (4, 8) form a stepped series of increments of Na + K in the perfusing water. Post-challenge mortality (PC) was obtained for Stacks 1-8 but not for 9-12.

	Treatment (mg/L)			PC mortality	Mean concentration of Ca^{2+}, Na^+, K^+ (mg/g dry weight) in alevins at sampling times (ATU, °C - days)																	
					(17 Jan) 755.3			(23 Jan) 790.5			(30 Jan) 828.9			(6 Feb) 864.9			(13 Feb) 898.5			(26 Feb) 959.0		
Stack	Ca^{2+}	$Na^+ + K^+$		(%)	Ca^{2+}	Na^+	K^+	Ca^{2+}	Na^+	K^+	Ca^{2+}	Na^+	K^+	Ca^{2+}	Na^+	K^+	Ca^{2+}	Na^+	K^+	Ca^{2+}	Na^+	K^+
	-	-	-																			
1	3.37	0.81	0.10	48.8																		
					1.864	1.471	4.655	1.384	2.051	6.138	0.612	1.830	6.418	1.743	3.630	7.473	3.248	4.004	8.640	4.211	2.200	7.850
5	3.39	0.85	0.12	74.6																		
	-	+	+																			
2	3.43	10.0	4.65	13.9																		
					-	-	-	1.130	2.631	6.133	0.585	2.238	6.912	1.852	3.818	7.249	2.611	2.894	7.465	4.054	3.271	8.389
6	3.39	9.82	4.62	42.5																		
	+	-	-																			
3	3.57	0.82	0.11	20.2																		
					1.715	0.774	2.634	1.268	2.155	5.577	0.876	1.904	7.327	1.628	3.320	6.215	2.738	3.454	7.276	4.385	1.547	7.918
7	3.52	0.79	0.10	39.1																		
	+	+	+																			
4	3.55	11.47	5.42	8.6																		
					1.837	1.819	5.242	1.311	2.305	5.846	0.943	1.930	6.870	1.571	3.426	7.725	2.712	3.460	7.694	4.270	4.141	9.702
8	3.49	10.42	5.05	1.8																		
	+	-	-																	(27 Feb)	(963.6)	
12	3.47	0.81	0.12	-	-	-	-	1.498	2.561	6.278	0.498	2.204	6.903	1.731	3.847	7.340	2.878	3.836	7.712	4.699	4.814	9.469
	+	-0.5	-0.5																			
9	3.52	3.75	2.42	-	-	-	-	1.417	2.369	5.393	1.576	1.899	6.197	1.747	3.438	7.666	2.665	3.826	8.493	-	-	-
	+	0	0																			
10	3.48	5.14	2.47	-	-	-	-	1.513	2.674	6.377	1.578	1.718	7.043	1.804	3.296	7.599	2.939	3.575	7.872	4.632	4.815	10.624
	+	+0.5	+0.5																			
11	3.50	8.40	3.98	-	-	-	-	1.545	2.423	5.963	0.859	2.262	7.555	1.636	3.718	7.547	2.835	3.596	8.512	4.699	4.814	9.469

stack. The main design (stacks 1-8) treats the appropriate trials of the original Al contrast as replicates. Hence, the eight stacks collapse into four sets of two stacks each around the contrasts for Ca^{2+} and $Na^+ + K^+$. The $Na^+ + K^+$ components were agricultural chemicals. These were dissolved with hatchery water to provide a stock solution, which was added to the appropriate stack water supplies at one of two levels (+1, -1) by peristaltic pumps. Ca^{2+} was added to the appropriate stacks by filling the upper three trays with crushed dolomite. As indicated earlier (Alderdice et al. 1987) the amount of Ca^{2+} going into solution was much less than anticipated, so that the (+1, -1) levels of Ca^{2+} in the design are very similar. A further four stacks (stacks 9-12) provided step increases in $[Na^+ + K^+]$ at the higher (+1) level of Ca^{2+} in a possible search for threshold concentrations of the monovalent cations.

Perfusion of the trays at the design levels began about one week before hatching and continued until the surviving alevins were ponded on 1-11 March 1986. The alevins in stacks 1-8 were challenged on 14 Feb. 1986 by inserting a tray of affected alevins above the test trays in each stack. Condition of the alevins was monitored and dead alevins were removed daily.

Samples of alevins were collected in the same manner as in 1983-84 (Alderdice and Rombough 1987). A sample consisted of 15 alevins, frozen and stored. For analysis, these were divided into five subsamples of three alevins each. Subsamples were oven-dried for 48 hr at 60°C. Na^+, K^+, and Ca^{2+} concentrations in tissues (mg/g dry weight) were determined as before (Alderdice and Rombough 1987) by AAS. U.S. National Bureau of Standards bovine liver (Standard Reference Material 1577a) was used to check the accuracy of Na^+ determinations. US N135 pine needles (SRM1575) was used in the same manner for K^+ and Ca^{2+}.

Initial bulk samples (15 alevins) were collected and diagnosed as "affected" or "normal" at the time of collection. Each data point in the series, for affected or normal alevins, is the mean of five subsample determinations.

RESULTS

Table 1 presents the modified design and the results of the analysis for Na^+, K^+, and Ca^{2+}. In the first eight stacks, stacks (1, 5) and (2, 6) occur at the background level of Ca^{2+}; stacks (3, 7) and (4, 8) are at the increased level of Ca^{2+}. Stacks (1, 5) and (3, 7) occur at background levels of $Na^+ + K^+$; those in stacks (2, 6), and (4, 8) are at the higher levels of $Na^+ + K^+$. The final four trials (stacks 12, 9, 10, 11) together with two of the first four sets of trials provide a series of step increments in $Na^+ + K^+$ at the higher Ca^{2+} level. These are stacks (3, 7, 12), (9), (10), (11), and (4, 8). The original data for the mean values of Na^+, K^+ and Ca^{2+} per sample of 15 alevins are listed in Appendix Table 1. The averages for the multiple samples (affected or

unaffected alevins) from the Appendix Table are listed in Table 1. The distributions of Na^+, K^+, and Ca^{2+} in the samples are illustrated in Figures 1-4.

SODIUM

Figure 1 illustrates the levels of tissue sodium in the lower [Na^+] trials (0.79-0.85 mg/L Na^+, stacks (1, 5) and (3, 7)) and the higher [Na^+] trials (9.8-10.47 mg/L Na^+, stacks (2, 6) and (4, 8)). The following observations are noted.

(1) Presence of affected alevins. There are more affected alevins in stacks (1, 5) and (2, 6) than in stacks (3, 7) and (4, 8). This occcurs even though stacks (1, 5) and (2, 6) differ by an order of magnitude in the [Na^+] of their perfusing water supplies.

(2) Rate of Na^+ uptake. In the lower [Na^+] trials (stacks (1, 5), (3, 7)), Na^+ uptake is approximately linear up to 900 ATU. There is a suggested reduction in uptake thereafter, although the data are limited and the latter trend is not seen in stack 12 (see Fig. 4), which is a replicate of these trials. In comparison, in the higher [Na^+] trials (stacks (2, 6), (4, 8)) Na^+ uptake increases linearly with time. The suggestion of rate-limited sodium uptake after 900 ATU remains, in which case the background [Na^+] (~0.8 mg/L) might be considered insufficient for normal Na^+ uptake in later alevin stages. However, this remains only a suggestion, based on the limited data.

(3) Timing, appearance of affected alevins. Most affected alevins were observed in the period of 790-900 ATU where there was no suggestion that Na^+ uptake was limited by environmental concentration.

(4) Sodium loss in affected alevins. Sodium loss in affected alevins in stacks (1, 5) and (2, 6) suggests the occurrence of massive sodium efflux from the tissues of affected alevins. Maximum loss occurred around 830 ATU, with some recovery thereafter at both lower and higher [Na^+] (stacks (1, 5) and (2, 6)).

POTASSIUM

Figure 2 illustrates the uptake of K^+ in the two pairs of lower [K^+] trials (0.10-0.12 mg/L K^+, stacks (1, 5) and (3, 7)) and higher [K^+] trials (4.62-5.42 mg/L K^+, stacks (2, 6) and (4, 8)). There are distinct similarities between the distributions of K^+, and those of Na^+ (Fig. 1).

(1) Presence of affected alevins. As for Na^+, there were more affected alevins in stacks (1, 5) and (2, 6) even though these trials differed by 4-5 orders of magnitude in K^+ concentration.

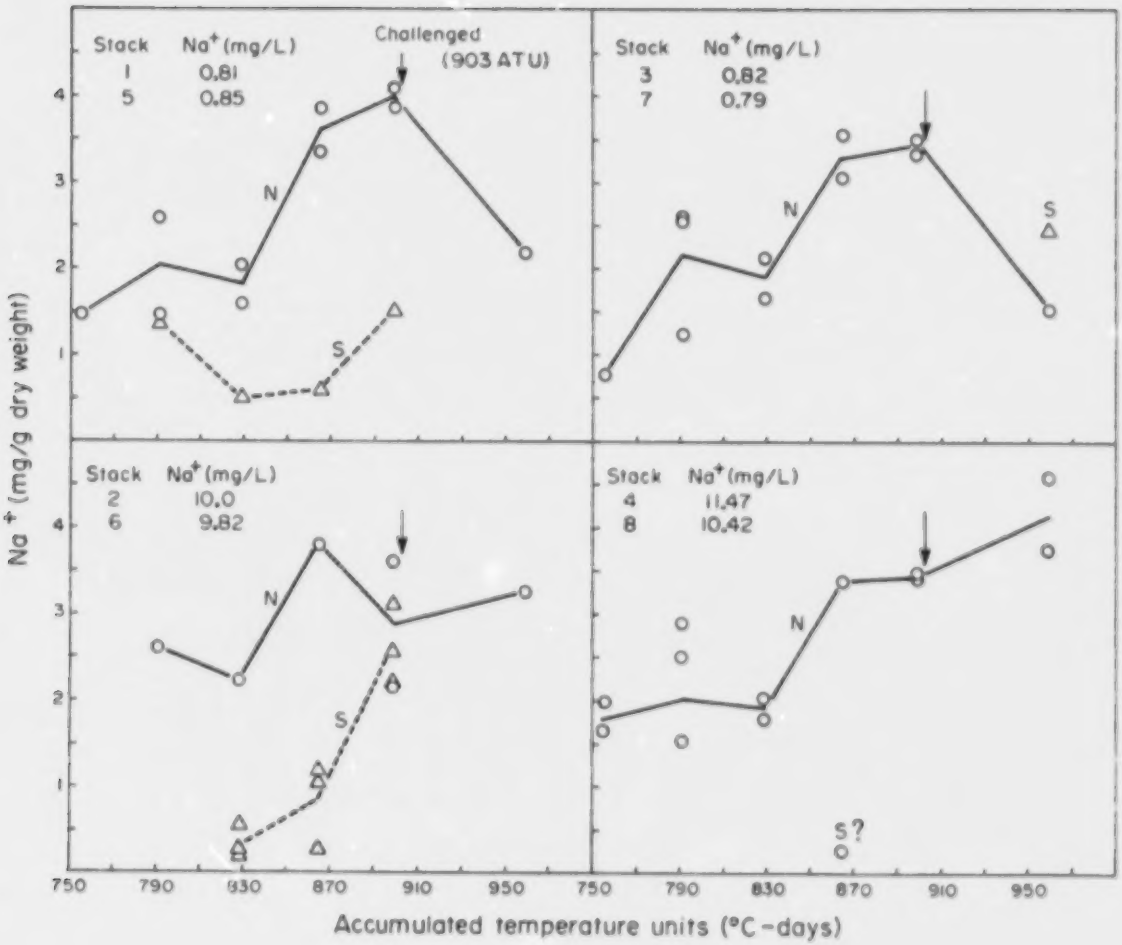

Fig. 1. Tissue concentrations of Na^+ (mg/g dry weight) in chinook alevins incubated in waters of differing mineral content at Chehalis Hatchery between 12 Dec. 1985 (11 days before hatch) and 1-11 March 1986 (ponding). Stacks (1,5) and (2,6): lower Ca level; stacks (3,7) and (4,8): higher Ca level. Circles -- normal alevins; triangles -- affected alevins. Arrows -- introduction of trays of affected alevins to challenge naive fish (14 Feb. 1986).

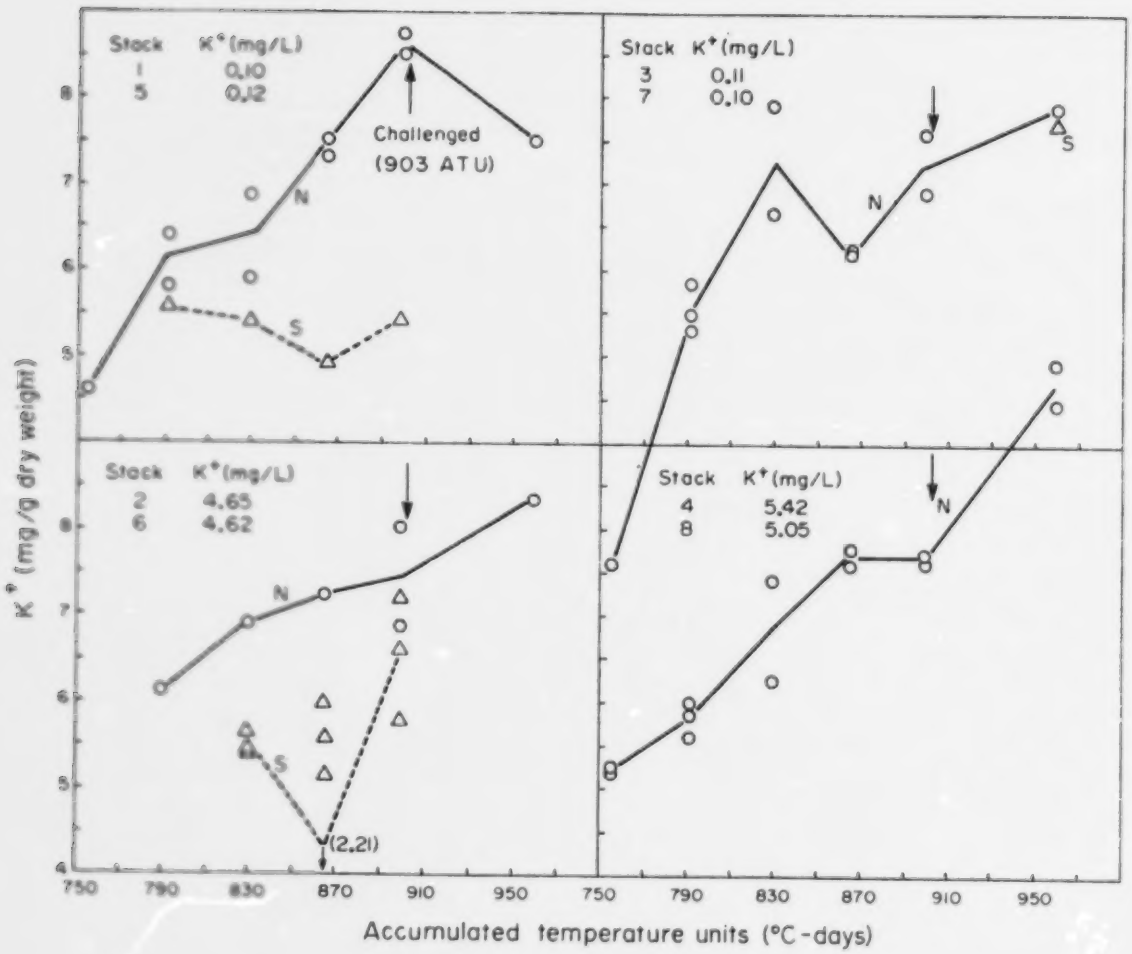

Fig. 2. Tissue concentrations of K^+ (mg/g dry weight) in chinook alevins incubated in waters of differing mineral content at Chehalis Hatchery between 12 Dec. 1985 (11 days before hatch) and 1-11 March 1986 (ponding). Stacks (1,5) and (2,6): lower Ca level; stacks (3,7) and (4,8): higher Ca level. For explanation of symbols, see Figure 1.

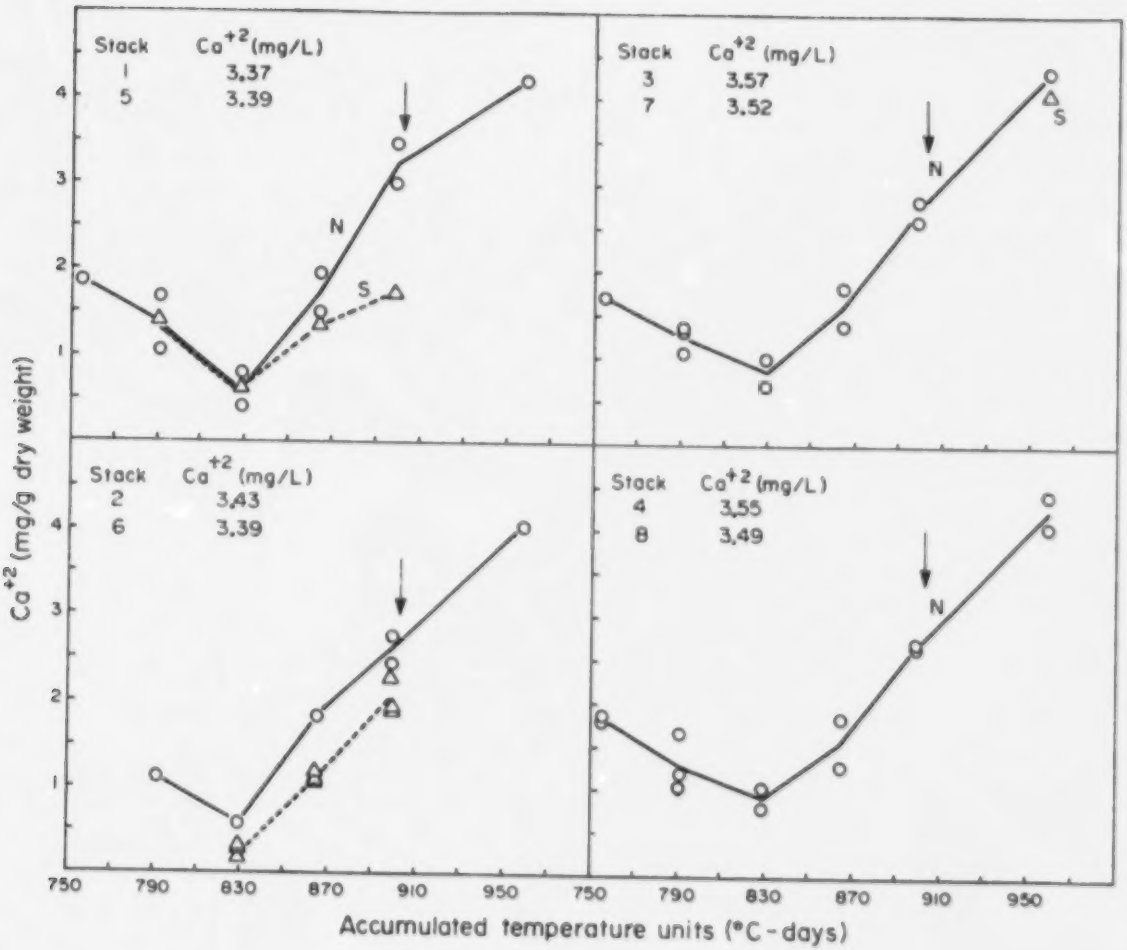

Fig. 3. Tissue concentrations of Ca^{2+} (mg/g dry weight) in chinook alevins incubated in waters of differing mineral content at Chehalis Hatchery between 12 Dec. 1985 (11 days before hatch) and 1-11 March 1986 (ponding). Stacks (1,5) and (3,7): lower $Na^+ + K^+$ level; stacks (2,6) and (4,8): higher $Na^+ + K^+$ level. For explanation of symbols, see Figure 1.

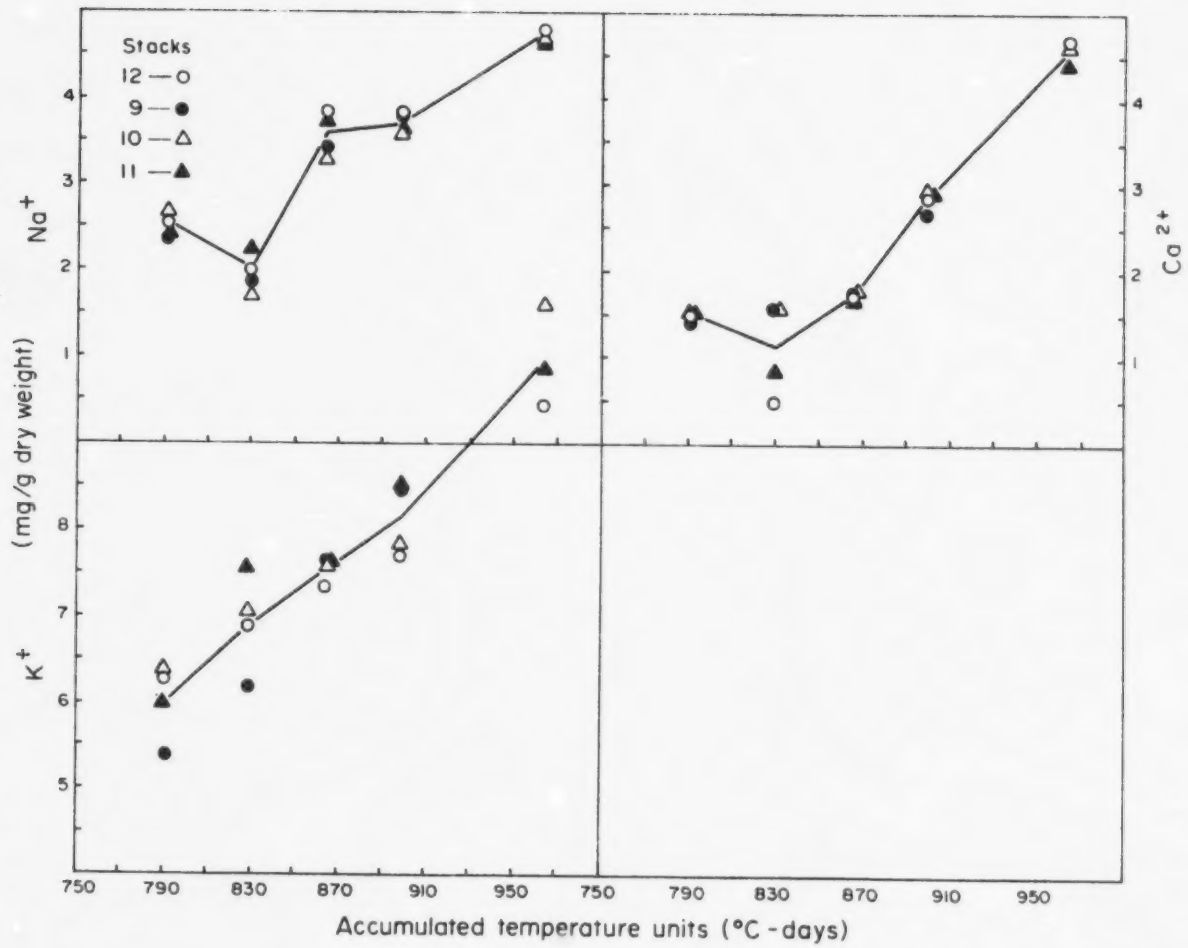

Fig. 4. Tissue levels of Na^+, K^+, and Ca^{2+} (mg/g dry weight) incubated in waters containing stepped increments of $Na^+ + K^+$ concentration at the higher level of Ca^{2+} (stacks 12,9,10,11) (see Table 1) at Chehalis Hatchery between 12 Dec. 1985 (11 days before hatching) and 1-11 March 1986 (ponding). These trials were not subject to challenge by affected alevins. For explanation of symbols, see Figure 1.

(2) Rate of K^+ uptake. In the lower [K^+] trials (stacks (1, 5) (3, 7)), K^+ uptake was approximately linear to 900 ATU. As with Na^+, there is a suggestion that uptake rate is reduced thereafter although this trend is not seen in stack (12), a replicate of stacks (3, 7). In comparison, in the higher [K^+] trial (stacks (2, 6), (4, 8)) K^+ uptake increased linearly with time. Again there is the suggestion that uptake of K^+ may be limited after 900 ATU by low environmental availability (0.10-0.12 mg/L), but this remains speculative because of the limited data.

(3) Timing, appearance of affected alevins. Most affected alevins were noted in the period of 790-900 ATU where, prior to 900 ATU, there was no suggestion that environmental level of K^+ might be limiting K^+ uptake.

(4) Potassium loss in affected alevins. Potassium loss in affected alevins (stacks (1, 5), (2, 6)) is indicative of abnormal net K^+ efflux from the tissues of affected alevins. Maximum loss occurred around 865 ATU, about 35 ATU later that when maximum Na^+ loss occurred. Some suggestion of more rapid recovery of K^+ after maximum loss is seen in the higher [K^+] trials (stacks 2, 6) although the variability in K^+ level in affected fish is high.

CALCIUM

It is pointed out again that the increase in Ca^{2+} achieved for the "high" Ca^{2+} trials (stacks 3, 7), (4, 8)) was much less than expected (3.40 mg/L Ca^{2+} in the "low" stacks, compared with 3.53 mg/L in the "high" stacks) (Fig. 3).

Presence of affected alevins. Almost all of the affected alevins were found in the trials at the lower level of [Ca^{2+}] (stacks (1, 5), (2, 6)), suggesting that [Ca^{2+}] had a greater influence on susceptibility to the unknown problem than either [Na^+] or [K^+]. This suggestion is speculative because of the minor difference in the lower and higher levels of Ca in the initial design; nevertheless, it is supported by the results of Alderdice et al. (1987) and by results of further studies at higher Ca levels (loc. cit., Addenda, 1986-87 trials).

Rates of Ca^{2+} uptake. In general, the trends in Ca^{2+} uptake are highly comparable in the four sets of two stacks compared. There is an initial period of Ca^{2+} decline from about 750 ATU until 830 ATU, followed by a linear rise in [Ca^{2+}] from 830 ATU until the trials were terminated (959 ATU). The initial decline could be related to the efflux of Na^+, and possibly K^+, which reached a maximum at 830 ATU. However, comparison with trials 12, 9, 10, 11 (Fig. 4), trials in which pathogen was concluded to be absent (Alderdice et al. 1987), shows the same dip in tissue [Ca^{2+}] at 830 ATU as occurred in the trials where pathogen was present (Fig. 3). On that basis, the decrease in Ca^{2+} uptake seen in the alevins exposed to pathogen (Fig. 3) at 830 ATU is most likely to represent normal variation in Ca^{2+} uptake rather than being a response to the presence of pathogen.

Timing, appearance of affected alevins. Most affected alevins were noted in the period from 790-900 ATU.

Calcium loss in affected alevins. The levels of tissue [Ca^{2+}] in the affected and unaffected alevins show no gross departure from each other, although [Ca^{2+}] in the affected alevins tends to be somewhat lower.

Stepped increments of $Na^+ + K^+$, trials 12, 9, 10, 11. For the series of stepped increases in concentration of $Na^+ + K^+$ in trials at the "high" Ca^{2+} level (stacks (3, 7, 12), (9), (10), (11), and (4, 8)) the results for trials 9-12 are shown in Figure 4. The remaining trials are shown in Figures 1-3. Most of the data lie between 790-900 ATU. In Figure 4, it appears that there are no obvious differences in uptake rates for the three elements that would suggest trends in uptake related to element concentration in the water supply. At background levels of $Na^+ + K^+$ (stacks 3, 7) and Ca^{2+} (stacks 1, 5), uptake rates are similar or slightly lower, but no trends could be suggested from the available data. Hence, the data provide no evidence of threshold environmental levels of the three elements that might influence uptake rates.

DISCUSSION

There is no substantial evidence from the tissue analyses of Na^+, K^+ or Ca^{2+} of strong associations between tissue levels of the three elements and alevin mortality. Alderdice et al. (1987) suspected that higher levels of the three elements in the water supplies led to a reduction in challenge mortality. At the higher hydromineral levels they did find that alevin mortality was reduced, but not eliminated. These observations appear to relate to the fact that the major proportion of affected fish was found in stacks (1, 5) and (2, 6), those stacks at the lower level of Ca^{2+} (3.37-3.43 mg/L). In this regard, most recent evidence (Alderdice et al. 1987, Addendum) shows that a substantial increase in Ca^{2+} in the water supply was related to a 5- to 6-fold increase in survival of challenged alevins (1986-87). Nevertheless, when the survivors were ponded in the low Ca^{2+} hatchery water supply, mortality increased. It appears that higher levels of Ca^{2+}, at least, provide some protection to the alevins. However, the measure of protection may have been sufficient only to postpone mortality until later in development.

It also is noted that the tissue levels of Na^+, K^+, and Ca^{2+} in the present trials generally are somewhat lower than those found by Alderdice and Rombough (1987) for Chehalis Hatchery chinook alevins in 1983-84.

Based on the evidence presented, water quality appears to be a peripheral, yet not insignificant, component of the Chehalis Hatchery chinook alevin problem. On all of the evidence, it is difficult not to conclude that a pathogen is most likely to be the primary source of the problem, in a stock with a genetic predisposition to increased susceptibility. Following this line of reasoning, the obvious osmoregulatory dysfunction seen, particularly with respect to Na^+ and K^+ effluxes in affected fish, could be a secondary response to the effects of the unknown pathogen.

Nevertheless, available evidence in the literature, in our opinion, will not as yet allow complete dismissal of the possibility that a physiological response to waters of low ionic strength could be an important component of the problem. Minimum levels of 10 mg/L Ca^{2+} and 1 mg/L Na^{+} are required for successful incubation and posthatching survival of brown trout (Salmo trutta) at pH 4.5 (Brown 1982). Requirements of these minerals in the development of Pacific salmon are not known, nor are they known at the usual pH of the Chehalis Hatchery water supply (6.4-6.6). A pH of 6.5 appears to be at or near a threshold, below which deleterious effects become increasingly obvious, particularly in low ionic strength waters. Most interesting is the study of Bradley and Rourke (1985) indicating that suboptimal [Na^{+}] can be involved with massive mortality in Salmo gairdneri juveniles at a pH of 6.6-7.1, and at Na^{+}, K^{+}, and Ca^{2+} concentrations very similar to those at Chehalis. Reduction in gross symptoms in the gills (filament swelling, edema, lamellar fusion) and mortality, began to subside about five days following Na^{+}, K, and Cl^{-} additions to the water supply, and gill morphology essentially was normal 27 days later (Bradley and Rourke 1985). The authors noted an inverse relation in affected fish between blood plasma [NH_4^{+}] and [Na^{+}] and suggested that low environmental Na^{+} levels could have reduced NH_4^{+} excretion by a Na^{+}/NH_4^{+} exchange mechanism. In their study, addition of 0.87 mM Na^{+} (20 µg/ml) to the water resulted in a 39% decrease in plasma [NH_4^{+}]

The Chehalis chinook alevin problem draws attention to the need for further information on the three components of the problem identified -- an unknown pathogen, stock differences in genetic susceptibility, and an involvement of waters of low ionic activity. From the viewpoint of salmonid enhancement, all three deserve further enquiry to increase the options for successful intervention in the manipulation of stocks and water supplies, either for hatchery operations or natural spawning.

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Appendix table 1. Concentrations of Ca^{2+}, Na^{+}, K^{+} (mg/g dry weight) in pooled samples of five alevins taken at intervals between hatching and swim-up. Treatments refer to stack numbers (Table 1). Mean tissue concentrations (Table 1) are based on sampled alevins that appeared normal and do not include those showing symptoms of illness(s).

Date 1986	ATU (°C days)	Sample no.	Ca^{2+}	Na^{+} (mg/g dry wt.)	K^{+}
Treatments 1, 5					
17 Jan	755.3	1	1.864	1.471	4.655
23 Jan	790.5	1	1.686	1.494	6.432
		2	1.082	2.608	5.844
		3(s)	1.399	1.358	5.583
30 Jan	828.9	1(s)	0.648	0.504	5.435
		2	0.808	2.054	6.914
		3	0.415	1.606	5.921
C Feb	864.9	1	1.514	3.875	7.593
		2(s)	1.482	0.639	4.940
		3	1.972	3.384	7.352
13 Feb	898.5	1	3.487	3.882	8.505
		2	3.009	4.125	8.774
		3(s)	1.736	1.530	5.429
26 Feb	959.0	1	4.211	2.200	7.850
Treatments 2, 6					
23 Jan	970.5	1	1.130	2.631	6.133
30 Jan	828.9	1	0.585	2.238	6.912
		2(s)	0.329	0.197	5.649
		3(s)	0.260	0.281	5.396
		4(s)	0.180	0.568	5.449
6 Feb	864.9	1(s)	1.110	1.047	5.160
		2	1.852	3.818	7.249
		3(s)	1.214	0.275	2.206
		4(s)	1.055	1.286	5.597
13 Feb	898.5	1(s)	2.310	3.120	7.238
		2(s)	1.916	2.587	6.637
		3(s)	1.954	2.286	5.802
		4	2.449	2.156	6.901
		5	2.773	3.631	8.028
26 Feb	959.0	1	4.054	3.271	8.389

Appendix table 1 (cont'd)

Date 1986	ATU (°C days)	Sample no.	Ca^{2+}	Na^{+}	K^{+}
			(mg/g dry wt.)		
Treatments 3, 7					
17 Jan	755.3	1	1.715	0.774	2.634
23 Jan	790.5	1	1.076	2.564	5.514
		2	1.350	1.257	5.350
		3	1.379	2.643	5.866
30 Jan	828.9	1	1.026	1.667	7.592
		2	0.726	2.141	6.702
6 Feb	864.9	1	1.403	3.565	6.215
		2	1.858	3.074	6.215
13 Feb	898.5	1	2.853	3.543	6.930
		2	2.623	3.365	7.621
26 Feb	959.0	1(s)	4.108	2.449	7.731
		2	4.385	1.547	7.918
Treatments 4, 8					
17 Jan	755.3	1	1.809	1.989	5.200
		2	1.864	1.649	5.284
23 Jan	790.5	1	1.195	2.505	5.626
		2	1.047	1.523	5.858
		3	1.690	2.888	6.054
30 Jan	828.9	1	0.833	2.047	7.449
		2	1.053	1.813	6.291
6 Feb	864.9	1	1.840	3.246	7.815
		2	1.302	0.296[a]	7.635
13 Feb	898.5	1	2.694	3.491	7.751
		2	2.731	3.428	7.636
26 Feb	959.0	1	4.459	4.507	9.937
		2	4.081	3.774	9.466

Appendix table 1 (cont'd)

Date 1986	ATU (°C days)	Sample no.	Ca^{2+}	Na^{+}	K^{+}
			(mg/g dry wt.)		
Treatment 9					
23 Jan	790.5	1	1.417	2.369	5.393
30 Jan	828.9	2	1.576	1.889	6.197
6 Feb	864.9	3	1.747	3.438	7.666
13 Feb	898.5	4	2.665	3.826	8.493
Treatment 10					
23 Jan	790.5	1	1.513	2.674	6.377
30 Jan	828.9	2	1.578	1.718	7.043
6 Feb	864.9	3	1.804	3.296	7.589
13 Feb	898.5	4	2.939	3.575	7.872
27 Feb	963.6	5	4.632	4.815	10.624
Treatment 11					
23 Jan	790.5	1	1.545	2.423	5.963
30 Jan	828.9	2	0.857	2.262	7.555
6 Feb	864.9	3	1.636	3.718	7.547
13 Feb	898.5	4	2.895	3.596	8.512
27 Feb	963.6	5	4.411	4.614	9.875
Treatment 12					
23 Jan	790.5	1	1.498	2.561	6.278
30 Jan	828.9	2	0.496	2.204	6.903
6 Feb	864.9	3	1.731	3.847	7.340
13 Feb	898.5	4	2.878	3.836	7.712
27 Feb	963.6	5	4.699	4.814	9.469

[a]Assumed in error, not included in calculations.

SUMMARY

by D. R. Harding

BACKGROUND

The Harrison River chinook is probably the most successful chinook stock in the Fraser River system. Spawners utilize the mainstem, sidechannels and sloughs downstream of Harrison Lake to the confluence of the Chehalis River, over a distance of about 10 linear kilometers (Hancock and Marshall 1985). Harrison chinook are known to be over 95 percent white-fleshed. They migrate to the sea as fry after a short rearing period (G. E. Rosberg and J. Millar, unpubl. data, Dept. Fish. Oceans, Vancouver, B.C.) and very few migrate north of Dixon Entrance (D. R. Harding, Dept. Fish. Oceans, unpubl. data, Vancouver, B.C.), thereby contributing mainly to Canadian troll and sports fisheries. It was because of the high productivity and the low level of foreign interception that this stock was chosen as the donor stock for enhancement at Chehalis and Chilliwack hatcheries.

Prior to the opening of Chehalis River Hatchery (CRH) in the fall of 1982, a few thousand Harrison River stock chinook salmon were incubated at Capilano Hatchery in 1970 and 1.5 million were incubated at Chilliwack River Hatchery in 1981. There were no indications at either hatchery (E. Stone and J. D. Buxton - pers. comm.) of the problems to come with this stock, nor did the usual symptoms appear at the Chilliwack hatchery in the 1982 to 1984 period when 1.5 to 2 million Harrison chinook eyed eggs were incubated each year from egg lots eyed at and split with the Chehalis hatchery.

The disease history of the Harrison River chinook stock (G. Hoskins, Pacific Biological Station, Nanaimo, pers. comm.) listed Ceratomyxa shasta as the most prevalent disease and includes some of the more common diseases such as furunculosis and bacterial gill disease. The nematode Anisakis was reported in adult samples. IHN virus was known to occur in the sockeye of Weaver Creek, a Harrison tributary and has been found in a few adult chinook. Harrison River spawning records report occasions of pre-spawning mortalities of chinook salmon, likely linked to C. shasta.

Because the previous attempts at incubation had been successful, the appearance of the symptoms (Traxler and Rankin 1987) in the 1982 brood at Chehalis was unexpected. Microbiological and histological examinations were negative (G. Hoskins, Pacific Biological Station, Nanaimo, pers. comm.) except that gill hyperplasia and clubbing were noted. These findings ruled out diseases other than those caused by unknown organisms or those very difficult to culture.

Water quality became a prime suspect because of the gill hyperplasia which is often associated with heavy metals and other pollution, and because of the success at Chilliwack where water hardness is usually 58-65 mg/L as $CaCO_3$. One factor that argued against problems of water quality was the random nature in which the syndrome struck individual trays in the stacks, sometimes missing complete headtanks. Water quality effects should be more

consistent, tending to have more effect either on the upper trays or the lower trays, depending on whether the stressor was in the water supply or was produced by the fish themselves. In the first two years the prevalence was low enough that the pattern of occurrence was not clear and it was not until a review of the first year's data that the suggestion of contagion was noted. It was hoped that the outbreak would be a one-time occurrence. However, careful recording of information on each tray was continued, especially the date of infection, if and when it occurred.

EARLY TRIALS TO IDENTIFY PROBLEM

For the first two brood years, attempts were made to remove or alleviate any possible mechanical or physical stresses. For example, the stacks were levelled and then tipped slightly forward to reduce chances of bubble formation under the eggtray screens; tray loads of alevins were moved out to keeper channels and intermediate rearing troughs in case something in the incubation room environment was causing a reaction; eggs were incubated on well water only, to reduce temperature and avoid pollutants such as possible spray residues in surface water. Various salts were used singly to reduce stress and increase hardness and pH. Water quality testing frequency was increased and disinfection and prophylactic treatments were done routinely.

Since none of the attempts seemed to reduce the problem, each Heath tray in 1984 was loaded with the eggs from one female and kept separate throughout incubation. In addition, 120 adults were examined and sampled for histology and pathology checks. Each adult was cross-referenced to its egg tray for correlation between adult condition and alevin mortality. Similar procedures were followed in subsequent years, however, no correlation has been found nor was there any apparent relationship with high egg infertility. The normal procedure at Chehalis is to load hatchery stacks from top to bottom before proceeding to the next stack. In 1984 most of the stacks were loaded horizontally, that is all position 2 trays were loaded before loading any position 3 trays. This was done to provide more oxygen to the more advanced alevins in the upper trays.

First evidence resulting from the addition of minerals (1984) to the water supply (Alderdice and Rombough 1987) suggested that Na^+, K^+ and Ca^{2+} could be involved in the alevin problem. In particular, it appeared that Na^+ and K^+ levels in the Chehalis Hatchery (Harrison) alevins were marginally lower than in Chilliwack alevins and substantially lower than levels in Capilano alevins. At that time it was uncertain whether the lower tissue ion levels were a cause or a result of the Chehalis alevin problem. Following this initial work NaCl was dripped into a headtank at a rate calculated to increase $[Na^+]$ by 4 mg/L beginning just before hatch. When symptoms appeared in a few trays, the drip rate was doubled. Finally, when this failed to stop the spread of symptoms, 20 mg/L of artificial marine salt was dripped into the headtank of the least developed alevins in the hope that some of the other ions in the marine salts might work in concert with NaCl to relieve the symptoms. Samples of alevins and of the water supplies also were analyzed for residues of organic pollutants such as herbicides and pesticides. Results from the water supplies were negative;

however, levels of known toxicants too low to cause the type of mortality experienced were found in the alevin samples (H. Rogers, West Vancouver, pers. comm.). Very little evidence could be found in the literature of levels of these chemicals that might be considered normal background concentrations for Pacific salmon. The following are the sample results:

chemical	µg/kg wet weight
PCP	3.57
DDE	18.22
DDD	4.62
DDT	5.63
PCB (1260)	26.03

First attempts to infect alevins of other stocks and species were made with the 1984 brood. Red chinook alevins from Capilano Hatchery (originally Big Qualicum stock) and Harrison-stock chinook alevins incubated and hatched at Chilliwack were "challenged" by being placed in the effluent of Chehalis-incubated Harrison-stock chinook alevins displaying symptoms. Both groups became infected but in neither case did the controls display symptoms. Even in these early attempts it appeared that the red-fleshed fish had a higher resistance since there were usually more survivors among the red-fleshed progeny. Red-fleshed chinook of Harrison River and Chehalis River origin have never been affected when incubated in Chehalis hatchery; however the numbers have been small and they have not been challenged. Coho alevins of various stocks challenged in the same manner have not been affected, however one tray of challenged chum alevins did display very similar symptoms. More recent attempts to duplicate that result were not successful and other trays of chum challenged at the same time were not affected.

Alevins from the 1984 brood were incubated to ponding in small numbers at the Pacific Biological Station in Nanaimo, B.C. without ill effect. Water quality there falls in the following ranges (J. Jensen, Nanaimo, pers. comm.):

Na	1.1 - 2.2 mg/L
Ca	4.3 - 7.6 mg/L
K	.15- .22 mg/L
Hardness	12 - 22 mg/L as CaCO3
pH	6.9

Up to this time all Harrison-stock chinook incubated at locations other than Chehalis had never displayed symptoms. It was not until the 1985 brood was incubated at the Chehalis Indian Band Hatchery (CIBH) upstream of the Chehalis River Hatchery with its own well water supply, that the symptoms were seen at another site. The CIBH has similar water quality to CRH and mortalities exceeded 50%. The egg lots used were shared with CRH where they also experienced heavy mortality. Small numbers of eggs also have been incubated at Rosewall Creek on Vancouver Island without symptoms appearing. Water quality there is similar to the highest levels at Nanaimo.

It is evident from the results of off-site incubation that water quality can influence the occurrence of the syndrome. With the high prevelance in the 1984 brood, it was also evident that some sort of contagion

was involved. The first appearance of the syndrome in any given stack was random by tray but after that, with very few exceptions, symptoms would appear in all the trays below an affected tray from 4 to 7 days later. It might also appear randomly in the trays above the first occurrence but would occur in the intervening trays within the following days. Thirdly, there appeared to be some difference in susceptibility related to stock or flesh colour. These pieces of information led to a three-pronged attack on the problem for the 1985 brood.

EXPANDED INVESTIGATIONS

The water quality experiment in 1985 had a few setbacks that led to a repeat of the experiment in 1986 with some important changes in design (Alderdice et al. 1987). A refinement of the aluminum measuring technique for the experiment resulted in a lowering of the detection limits. Subsequently it was determined that aluminum was not being added to the water within the hatchery and that dissolved levels were below those considered harmful. The original design considered water quality as the sole source of the problem and the experiment determined that this was not the case. Infection was left as an uncontrolled variable until a challenge was added late in the alevin stage. Unfortunately, the challenge was made much later than the time when infection usually occurred and there was difficulty in obtaining sufficient infected alevins to provide a controlled challenge. However, the results gave good indications of which parameters were important for the conduct of a more rigorous experiment on the 1986 brood.

The infectious agent experiments (Traxler and Rankin 1987) determined that UV irradiation with filtering could stop the transmission of the condition from infected alevins to others placed in their effluent. This finding then supported the conclusion that the agent was not in the water supply, since UV irradiation with filtering of the water supply to a stack had no effect on the occurrence of the infection in that stack. Also the agent was not on the surface of the egg, as surface-disinfection of eggs at fertilization or at the eyed-stage did not stop the alevins from becoming infected. These two parts of the experiments also demonstrated that contagion occurred between trays of alevins. The third major part of these experiments added to the evidence that a red-fleshed alevin from another stock was less susceptible than a Harrison white chinook and did not become infected unless placed in the effluent of an infected tray.

The genetics experiments (Withler 1987) determined that the Harrison stock was more susceptible than the Capilano stock and that susceptibility was determined more by the male parent, especially where the die-off was rapid. The groups that were incubated at Rosewall Creek did not become infected.

The genetics experimentation was not continued in 1986 and the 1986 infectious agent studies are incomplete and will be reported elsewhere. Preliminary results tend to support previous findings (G. Traxler, pers. comm.).

The 1986 water quality experiments (Alderdice et al. 1987 addendum) featured a challenge made as uniform as possible by a pooling of infected alevins. Each of three pooled groups was used above and below the challenge group. That symptoms appeared in some of the unchallenged trays and not in the unchallenged controls demonstrates the previous findings that the occurrence of the infection is "hit and miss". Even if the eggs of one female are divided into subsamples, the symptoms may not appear in every subsample group.

The 1986 challenge may have been overdone in that the density of disease organisms in the effluent of a challenge tray may have greatly exceeded that found in a tray with only a few infected alevins. If there were only a few infected alevins per tray then the levels of minerals added might have been sufficient to prevent the outbreak from becoming epidemic. The water quality in the stack with the highest levels of added minerals is similar to that at Rosewall and Nanaimo where outbreaks have not occurred, yet mortality in that stack was near 80%. Even though survival in this group was increased fivefold, it is difficult to reconcile that mortality with the absence of symptoms at the other locations. Levels of these minerals are even higher at Chilliwack so one could expect higher protection there based on the surface analysis plot (ibid.).

As a result of the studies conducted to date we can arrive at a number of conclusions, the most important being that contagion occurs. Every challenge that has been made has been successful and it appears that the alevins may be susceptible for a longer time than originally thought, that is, even beyond swim-up. The addition of minerals in combination provides some protection although not as much as desired at the level of challenge tested. The agent has not been discovered and thus levels cannot be measured, so the level of protection needed is not known. There are interstock differences in susceptibility, more of which seems due to the male parent.

One finding important to the hatchery is that the agent is not in the water supply, neither in the wells nor in the surface supply. Also it is not on the egg. Therefore we have to conclude that the agent is passed from the parent into some of the eggs. After hatch it is transmitted from infected alevins to others.

We have found no deleterious levels of aluminum, heavy metals, pesticides, or herbicides and have ruled out the effects of temperature, density and many possible mechanical/physical causes. No known pathogens have been found and, other than the gill hyperplasia and blood abnormalities, no target organ or tissue has been identified.

The low number of immature red blood cells suggests an impairment of cell production sites; however, no damage has been detected. A low total red cell count might be responsible for the anaemic appearance. The thickened gill epithelium suggest an irritant at the gills and observed gill anomalies could be a response to reduced ion and gas transfer across the thickened epithelium. Anaemia and reduced gas transfer would result in the lethargy observed among affected alevins. This speculation on our observations would suggest that the gill tissue is a probable site for the agent of contagion. Because parasites, bacteria and known viruses have been ruled out as causes, an unknown virus or one of the little known organisms intermediate to viruses

and bacteria is expected to be the cause. A search for a probable cause is continuing using the electron microscope; other methods of isolating or cultivating the organism have not been successful.

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